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DOCUMENT-IDENTIFIER: US 6063283 A
TITLE: Method for analyzing a sample by using a liquid chromatograph

DEPR:
In the present invention, porous media commonly used in liquid chromatography
such as silica gel, alumina, porous glass beads, zeolite, hydroxyapatite and
graphite may be used as the packing material of the column 40. Alternatively,
it is possible to use **composite** powders formed of a **resin core covered by**
inorganic powders such as silica gel, titanium dioxide, hydroxyapatite, and the
like. On may use materials such as polyamides, acrylic resins, polyvinyl
alcohols, and the like, for the resin core.

CCXR:
210/198.2

DOCUMENT-IDENTIFIER: US 6054047 A
TITLE: Apparatus for screening compound libraries

BSPR:
Preferably, the solid phase support used in this invention is selected from the group consisting of **resin beads, glass beads, silica** chips, silica capillaries and agarose.

DEPR:
When employing the apparatus of this invention, the target receptor is optionally bound or coupled to a solid support. Preferably, the target receptor is covalently bound or coupled to the solid support. However, in some cases, such as when whole cells or organisms are employed as the target receptor, the cells or organisms may be contained within the column by using, for example, a porous frit at the outflow end of the column. Supports for receptors are well-known in the art and many are commercially available. Any such conventional support may be used in this invention. Representative supports include, by way of illustration, **resin beads, glass beads, silica** chips and capillaries, agarose, and the like. When silica capillaries are used as the solid support, the target receptor is bound directly to the walls of the column. Preferred solid supports for use in this invention include porous resin beads. A particularly preferred solid support is porous polystyrene-divinylbenzene polymer beads, such as POROS beads (available from Perseptive Biosystems, Framingham, Mass.).

DEPR:
When employing multiple columns, each column is typically monitored for a brief period of time before switching to the next column. For example, with a quadrupole mass spectrometer, each column is typically monitored sequentially for a period of about 0.5 seconds to about 10 seconds, preferably

for about 1
second to about 5 seconds, before switching to the next column. The effluent
from each column is analyzed as described herein using mass spectrometry.
Generally, a single running file is used to collect all of the data from the
multiple column thereby generating a **composite** total ion chromatogram.
Subsequently, separate total ion chromatograms for each column are recreated by
synchronizing column switching with mass spectrometry data acquisition.

CLPR:
16. The apparatus of claim 14, wherein the solid phase support is selected
from the group consisting of **resin beads, glass beads, silica** chips, silica
capillaries and agarose.

CCOR:
210/198.2

DOCUMENT-IDENTIFIER: US 6045697 A
TITLE: Passivated porous polymer supports and methods for the preparation and
use of same

BSPR:
Several previous investigators have sought to passivate various microporous
media including membranes and particulate chromatographic supports by applying
thin surface coatings to inorganic or organic/polymeric substrates. For
example, Steuck, in U.S. Pat. No. 4,618,533, discloses a porous polymeric
membrane substrate fashioned from a thermoplastic organic polymer upon which a
permanent coating is grafted and/or deposited on the entire membrane surface.
The polymerization and crosslinking of the polymerizable monomer upon and
within the porous membrane substrate is performed in such a way that a thin
coating is deposited upon the entire surface of the porous membrane, including
the inner pore walls. Significantly, the porous configurations of the coated,
composite membrane structures claimed by Steuck are essentially identical to
those of the corresponding uncoated porous membrane substrates, implying that
the polymer of Steuck is applied as a thin surface layer or coating that does
not interfere with the porosity or flow properties of his **composite** membranes.
Moreover, Steuck does not disclose the concept of a "passivating layer" or the
use of monomers capable of functioning as "passivating" monomers within the
meaning of the present invention as discussed in more detail below.

BSPR:
While it has been discovered that this process of depositing protective polymer
coatings upon the porous surfaces of mineral oxide (and particularly silica)
matrices can significantly stabilize these materials by sharply reducing their

rates of chemical leaching, the approach has the important disadvantage of rendering the porous surfaces of the coated and protected matrices hydrophobic and thus prone to cause excessive non-specific binding of proteins by adsorption. (This is precisely the same problem noted above in connection with entirely polymeric porous support matrices.) However, this problem can be successfully addressed by the methods of the present invention in the same way as the non-specific binding of strictly polymeric support matrices can be reduced--i.e., by passivation in a process of oriented polymerization. More particularly these **composite** chromatographic supports (i.e., supports comprised of mineral oxide substrates that have been stabilized by the application of thin protective polymer coatings) can be passivated against excessive non-specific binding by incorporating passivating ("neutralizing") monomers capable of associating with and consequently deactivating innate nonpolar hydrophobic groups exposed on the matrix surface. The passivating monomers useful in this embodiment of the present invention adsorb upon (and consequently cover) the hydrophobic groups on the surface by virtue of their containing long-chain saturated hydrocarbons, olefinic hydrocarbon groups, aromatic groups, or like hydrophobic domains that interact with and become appreciably bound to their hydrophobic counterparts on the matrix surface as a consequence of the hydrophobic-hydrophobic interaction existing between them.

Typically, the present invention utilizes base matrices having the following characteristics: an initial average particle size ranging from about 5 to about 1000 .mu.m; an initial porous volume ranging from about 0.2 to about 2 cm.sup.3 /gram; an initial surface area ranging from about 1 to about 800 m.sup.2 /gram; and an initial pore size ranging from about 50 to about 6000 angstroms.

Preferably, the base matrix is characterized by: an initial average particle size ranging from about 10 to about 300 .mu.m, although passivated supports having narrow particle size ranges, such as about 15-20 .mu.m, about 15-25 .mu.m, about 30-45 .mu.m, about 50-60 .mu.m, about 80-100 .mu.m, and about 100-300 .mu.m, are most preferred. Preferred ranges for other characteristics include an initial porous volume ranging from about 0.8 to about 1.2 cm.sup.3 /gram; an initial surface area ranging from about 10 to about 400 m.sup.2 /gram; and an initial pore size ranging from about 1000 to about 3000 angstroms. The density of the porous solid matrix obviously varies with its chemical nature, being higher for mineral oxide (e.g., silica) substrates and lower for polymeric ones (e.g., polystyrene).

DEPR:
It is thus demonstrated that the level of non-specific adsorption for lysozyme (a strong cationic protein) is high when the MAPTAC is absent. The non-specific adsorption for silica with large surface areas (X 075, 100m.sup.2 /g) is higher (55 mg/mL of **resin) than the non-specific adsorption for silica** X 015 (25 m.sup.2 /g; 15 mg/mL of resin). A certain proportionality exists between the surface area and the original level of non-specific absorptions. The amount of MAPTAC to decrease the level of non-specific adsorption down to zero is also proportional to the surface area available: 1.5% of MAPTAC is necessary with silica X 015 (25 m.sup.2 /g) whereas at least 6% is necessary to passivate silica X 075 (100 M.sup.2 /g).

DEPR:
The passivated resin is then recovered by filtration. The oil is eliminated with an extensive washing with water containing 0.1-0.5% of a non-ionic detergent and then stored in a saline buffer at neutral pH. The product resin

shows very similar ion-exchange characteristics as those described in Example 2. Additionally, its sensitivity in strong alkaline media is much improved as measured by its weight loss after one night of contact with 0.5 M sodium hydroxide. The passivated resin of this example lost only about half as much weight as an anionic **resin prepared from silica** having an unprotected surface area.

DEPR:
The polymer-silica **composite** was then washed several times with water, with diluted hydrochloric acid and with diluted sodium hydroxide solution (0.2M). After neutralization the ion exchanger was used for protein separation. The number of anionic groups per mL of wet materials was 110 .mu.eq and sorption capacity for cationic proteins (e.g., lysozyme) was 120 mg/mL of resin.

DEPC:
Preparation of an Anion-Exchange **Resin Using a Surface-Protected (i.e., Pre-coated) Silica** Passivated Porous Support

DEPC:
Determination of Ion-Exchange and Protein Sorption Capacity of Preparation of Anion-Exchange **Resins Based on Passivated Porous Silica** Support of Different Surface Areas

CCXR:
210/198.2

DOCUMENT-IDENTIFIER: US 5906734 A
TITLE: Passivated porous polymer supports and methods for the preparation and
use of same

BSPR:
Several previous investigators have sought to passivate various microporous
media including membranes and particulate chromatographic supports by applying
thin surface coatings to inorganic or organic/polymeric substrates. For
example, Steuck, in U.S. Pat. No. 4,618,533, discloses a porous polymeric
membrane substrate fashioned from a thermoplastic organic polymer upon which a
permanent coating is grafted and/or deposited on the entire membrane surface.
The polymerization and crosslinking of the polymerizable monomer upon and
within the porous membrane substrate is performed in such a way that a thin
coating is deposited upon the entire surface of the porous membrane, including
the inner pore walls. Significantly, the porous configurations of the coated,
composite membrane structures claimed by Steuck are essentially identical to
those of the corresponding uncoated porous membrane substrates, implying that
the polymer of Steuck is applied as a thin surface layer or coating that does
not interfere with the porosity or flow properties of his **composite** membranes.
Moreover, Steuck does not disclose the concept of a "passivating layer" or the
use of monomers capable of functioning as "passivating" monomers within the
meaning of the present invention as discussed in more detail below.

BSPR:
While it has been discovered that this process of depositing protective polymer
coatings upon the porous surfaces of mineral oxide (and particularly silica)
matrices can significantly stabilize these materials by sharply reducing their

rates of chemical leaching, the approach has the important disadvantage of rendering the porous surfaces of the coated and protected matrices hydrophobic and thus prone to cause excessive non-specific binding of proteins by adsorption. (This is precisely the same problem noted above in connection with entirely polymeric porous support matrices.) However, this problem can be successfully addressed by the methods of the present invention in the same way as the non-specific binding of strictly polymeric support matrices can be reduced--i.e., by passivation in a process of oriented polymerization. More particularly, these **composite** chromatographic supports (i.e., supports comprised of mineral oxide substrates that have been stabilized by the application of thin protective polymer coatings) can be passivated against excessive non-specific binding by incorporating passivating ("neutralizing") monomers capable of associating with and consequently deactivating innate non-polar hydrophobic groups exposed on the matrix surface. The passivating monomers useful in this embodiment of the present invention adsorb upon (and consequently cover) the hydrophobic groups on the surface by virtue of their containing long-chain saturated hydrocarbons, olefinic hydrocarbon groups, aromatic groups, or like hydrophobic domains that interact with and become appreciably bound to their hydrophobic counterparts on the matrix surface as a consequence of the hydrophobic-hydrophobic interaction existing between them.

Typically, the present invention utilizes base matrices having the following characteristics: an initial average particle size ranging from about 5 to about 1000 microns; an initial porous volume ranging from about 0.2 to about 2 cm.sup.3 /gram; an initial surface area ranging from about 1 to about 800 m.sup.2 /gram; and an initial pore size ranging from about 50 to about 6000

angstroms. Preferably, the base matrix is characterized by: an initial average particle size ranging from about 10 to about 300 microns, although passivated supports having narrow particle size ranges, such as about 15-20, about 15-25, about 30-45, about 50-60, about 80-100, and about 100-300 microns, are most preferred. Preferred ranges for other characteristics include an initial porous volume ranging from about 0.8 to about 1.2 cm.sup.3 /gram; an initial surface area ranging from about 10 to about 400 m.sup.2 /gram; and an initial pore size ranging from about 1000 to about 3000 angstroms. The density of the porous solid matrix obviously varies with its chemical nature, being higher for mineral oxide (e.g., silica) substrates and lower for polymeric ones (e.g., polystyrene).

DEPR:
The passivated resin is then recovered by filtration. The oil is eliminated with an extensive washing with water containing 0.1-0.5% of a non-ionic detergent and then stored in a saline buffer at neutral pH. The product resin shows very similar ion-exchange characteristics as those described in Example 2. Additionally, its sensitivity in strong alkaline media is much improved as measured by its weight loss after one night of contact with 0.5 M sodium hydroxide. The passivated resin of this example lost only about half as much weight as an artionic **resin prepared from silica** having an unprotected surface area.

DEPR:
The polymer-silica **composite** was then washed several times with water, with diluted hydrochloric acid and with diluted sodium hydroxide solution (0.2 M). After neutralization the ion exchanger was used for protein separation. The number of anionic groups per ml of wet materials was 110 .mu.eq and sorption

capacity for cationic proteins (e.g., lysozyme) was 120 mg/ml of resin.

DEPC:
Preparation of an Anion-Exchange **Resin Using a Surface-Protected (i.e., Precoated) Silica** Passivated Porous Support

DEPC:
Determination of Ion-Exchange and Protein Sorption Capacity of Preparation of Anion-Exchange **Resins Based on Passivated Porous Silica** Support of Different Surface Areas

DEPC:
Preparation of a Cation-Exchange **Resin Using a Surface--Protected (i.e., Precoated) Porous Silica** Support

CCOR:
210/198.2

DOCUMENT-IDENTIFIER: US 5906747 A
TITLE: Separation of molecules from dilute solutions using **composite**
chromatography media having high dynamic sorptive capacity at high flow rates

TTL:
Separation of molecules from dilute solutions using **composite** chromatography
media having high dynamic sorptive capacity at high flow rates

BSPR:
This invention relates to chromatography media and their use. In particular,
composite media are disclosed which are characterized by a reversible high
sorptive capacity. These media may be passivated to prevent non-specific
adsorption of or interaction with biomolecules such as proteins, oligopeptides,
polysaccharides, and nucleotides. The **composite** media of the present invention
exhibit characteristics that are highly desirable in chromatographic
applications, such as high porosity, physical rigidity, high charge density,
and chemical stability under a variety of extreme conditions, and may be used
advantageously with especially dilute feed streams in high-flow, high-efficiency mass transfer chromatographic techniques that can be carried
out in a fluidized-bed, packed-bed, or other mode of operation.

BSPR:
Several previous investigators have sought to passivate various microporous
media including membranes and particulate chromatographic sorbents by applying
thin surface coatings to inorganic or organic/polymeric substrates. For
example, Steuck, in U.S. Pat. No. 4,618,533, discloses a porous polymeric
membrane substrate fashioned from a thermoplastic organic polymer upon which a
permanent coating is grafted and/or deposited on the entire membrane surface.
The polymerization and crosslinking of the polymerizable monomer upon and

within the porous membrane substrate is performed in such a way that a thin coating is deposited upon the entire surface of the porous membrane, including the inner pore walls. Significantly, the porous configurations of the coated, **composite** membrane structures claimed by Steuck are essentially identical to those of the corresponding uncoated porous membrane substrates, implying that the polymer of Steuck is applied as a thin surface layer or coating that does not interfere with the porosity or flow properties of his **composite** membranes. Moreover, Steuck does not disclose the concept of a "passivating layer" or the use of monomers capable of functioning as "passivating" monomers within the meaning of the present invention as discussed in more detail below.

BSPR:
The present invention provides a method for the separation of biological molecules by chromatography. The method of the invention comprises the steps of passing a sample containing a mixture of biological macromolecules including a biological macromolecule of interest through a column packed with a **composite** media and recovering the biological macromolecule of interest from the sample. The **composite** media which characterizes the present invention provides a larger dynamic capacity for a biological macromolecule at low initial feed concentrations, for example, those less than about 2 milligrams per milliliter, than the dynamic capacity provided by the same media for the same macromolecule at higher concentrations, for example, those higher than about 2 milligrams per milliliter. The media preferably provides a larger dynamic capacity for said macromolecule at initial feed concentration in the range of about 10 micrograms per milliliter to about 2 milligrams per milliliter than the dynamic capacity provided by the same media for the same macromolecule at initial feed

concentrations in the range of about 2 milligrams per milliliter to about 100 milligrams per milliliter. In a preferred embodiment of the present invention, the sample to be subjected to the separation procedure may have an initial concentration of the biological macromolecule of interest of less than about 2 milligrams per milliliter.

BSPR:
In accordance with the present invention, the media may be a **composite** media that comprises a porous support comprising voids containing a polymeric network, wherein the **composite** media provides a value of the flux enhancement factor E greater than about 3, preferably greater than about 20, as determined by the equation ##EQU1## in which D.sub.s is the experimentally measured effective intraparticle diffusivity of a molecule of interest, q.sub.0 is the equilibrium concentration of said molecule within the media particles at equilibrium with C.sub.0, D.sub.f is the diffusivity in free solution of said molecule, .epsilon..sub.p is the fractional void volume of the porous support of the **composite** media, .tau. is the tortuosity of the porous support of the **composite** media, and C.sub.0 is the concentration of said molecule in the feed solution, and is preferably at least about 1 microgram per milliliter, more preferably at least about 10 micrograms per milliliter.

BSPR:
The **composite** media may be selected to be a chromatographic media, more preferably, ion-exchange chromatography media, so that said biological macromolecules can be separated by (ion-exchange) chromatography. The chromatography media is preferably one that provides an intraparticle diffusional flux that is faster, for instance at least about 30% faster, than the diffusional flux of the biological macromolecules in solution.

DEPR:
The present invention provides a **composite** media for the separation of biological molecules by chromatography, such as ion-exchange chromatography. As noted above, the media provides a flux enhancement factor E of greater than 3 wherein ##EQU2## In a further refinement, the media provides a value for a second figure of merit or flux enhancement factor E* of greater than 3, wherein ##EQU3## in which D.sub.s is the effective intraparticle diffusivity of the biological molecule of interest, .tau. is the tortuosity factor of the **composite** media, D.sub.f is the diffusivity in free solution of the biological molecule, and .epsilon..sub.p * is the species-dependent inclusion porosity of the **composite** media (Athalye, A. M. et al., J. Chromatocraphy, 589 (1992) 71-85).

DEPR:
The terminology "**composite** media" is intended to cover all combinations of physical (or support) structures or compounds, including mineral oxide matrices, mineral oxide matrices whose interior and exterior surfaces are substantially covered by a thin protective polymer surface coating, metal matrices, and polymeric matrices such as polystyrenes, with chromatographic resins such as those made from chemical substances known to be useful for the preparation of chromatographic separation adsorbents, including polymerized vinyl monomers that contain chromatographically active substituents. The constituents of the **composite** media can include structures and chromatographic resins made separately or formed together, such as in block copolymers.

DEPR:
An important figure of merit that facilitates understanding the present invention is the formula for flux enhancement factor ##EQU4##

wherein D.sub.s
is the experimentally determined effective intraparticle diffusivity of the
biological molecule to be separated, .tau. is the tortuosity factor of the
porous chromatography support, D.sub.f is the diffusivity in free solution of
the biological molecule, and .epsilon..sub.p is the void volume of the
composite support matrix measured in the absence of a polymeric gel filling the
pores. D.sub.s is defined in such a way that the driving force for diffusion
in the particle is the gradient in the total protein concentration at that
point in the particle, not just the gradient in "free" or "unbound" protein
concentration. Thus the flux enhancement factor E is determined by both the
characteristics of the chromatography medium e.g. (.tau. and .epsilon..sub.p)
and the characteristics of the biological molecule to be separated (e.g.
D.sub.s and D.sub.f). The variable D.sub.s depends upon the biological
molecule as well as on the composite media. This flux enhancement factor E
represents the ratio of (i) the intraparticle diffusional solute flux to (ii)
the intraparticle flux of solute that would be expected to occur if the pores
of the composite media support were devoid of the polymeric network.

DEPR:
The second flux enhancement factor E* takes account of the steric effects of
the gel residing within the pores of the composite media. This flux
enhancement factor E* is the ratio of (i) the intraparticle diffusional solute
flux to (ii) the intraparticle flux of solute that would occur if the pores of
the composite media support were filled with a polymeric network identical to
that present in the composite media of the invention save for its being
incapable of interacting electrostatically or chemically with the solute of
interest. The steric effects of the gel decrease the effective

area open for diffusion in the **composite** media. A decrease in effective area for diffusion would, in a conventional media, significantly inhibit diffusion in the gel. In the present invention, however, this steric effect of the gel is overcome by the favorable partitioning of solute into the gel; that is, the high intraparticle solute concentration gives rise to an increase in the total driving force for diffusion in the **composite** media and thus an increase in the total flux into the particle. It may be noted that E and E* account for the fact that the biological macromolecule must diffuse by a geometrically tortuous route in order to penetrate the particle. Thus, in comparing the enhancement of intraparticle flux with that in free solution, E and E* account for the longer diffusional distance with the tortuosity parameter .tau.. Values for .tau. generally vary between 2 and 6. A value for tortuosity of 2 has been adopted for calculations of E and E* presented below, because such a value typical of many chromatographic media, and because adoption of a tortuosity value of 2 results in calculated E and E* values that are conservative.

DEPR:
The flux enhancement factors E and E* defined above are designed so as to capture the unexpectedly high mass transfer performance (e.g., intraparticle solute fluxes) of the **composite** media of the present intention. In particular, the first flux enhancement factor E is meant to compare the observed rate of intraparticle solute mass transfer with the rate of mass transfer that would be predicted were the solute to be diffusing into the "empty" (e.g., gel-free) porous support particle from which the **composite** media of the present invention is fashioned. One of ordinary skill might reasonably expect the solute to diffuse into the gel-containing **composite** particle no more

rapidly than it
would diffuse into the "empty" support particle; this expected rate of mass
transfer would be proportional to the solution-phase solute concentration
driving force C.sub.0 and to an effective intraparticle solute diffusivity for
such a gel-free particle given by the following expression:
##EQU5## Inasmuch
as E is the ratio of a term proportional to the actual intraparticle flux
(i.e., D.sub.s .cndot.q.sub.0) to a comparable term proportional to the flux
that would be predicted for a gel-free porous support particle, then --absent
other considerations (i.e., steric exclusion by the gel)--the expected value of
E would be of order unity or less. In contrast, the flux enhancement factor E
achievable through the present invention can be substantially greater than
unity--and significantly greater than the E values provided by prior-art
media--as shown in more detail below.

DEPR:
The degree of flux enhancement achievable through the present invention is
particularly unexpected when the steric exclusion of the polymer network or gel
present within the pores of the **composite** media support is taken into account
in the prediction of the expected intraparticle solute flux. In particular,
the presence of the gel within the support particle's pores reduces the porous
volume (or, alternatively, the effective area for diffusion) that is accessible
to molecules diffusing within it. It is this steric effect of the gel in
reducing this expected intraparticle flux which the second flux enhancement
factor E* is designed to capture. In particular, one of ordinary skill would
expect the solute to diffuse into the **composite** particle no more rapidly that
it would diffuse into a support particle filled with a non-interactive gel; in
this case, the expected rate of mass transfer would again be proportional to

the solution-phase solute concentration driving force C.sub.0 --but the
effective intraparticle diffusivity would be smaller, as given by the following
expression: ##EQU6## where .epsilon..sub.p * is the species-dependent inclusion
porosity. Inasmuch as E* is the ratio of a term proportional to the actual
intraparticle flux (i.e., D.sub.s .cndot.q.sub.0) to a term proportional to the
predicted or expected intraparticle flux taking steric exclusion of solute by
the gel into account, one of ordinary skill might reasonably anticipate that
composite media would provide E* values of order unity or lower. However, the
composite media of the present invention are characterized by much larger E*
values that differentiate them from prior-art **composite** media.

DEPR:
The parameter .tau. is the tortuosity factor characteristic of the skeletal
matrix of the **composite** media support particle. The parameter .tau. is
related to the additional distance that a solute has to diffuse in the tortuous
pores of the support as compared to how far it would have to diffuse in
"straight" pores or in free solution. Because the pore walls of the **composite**
media of the support particle do not allow diffusion through them, the solute
cannot diffuse directly from some point A to some point B; instead the solute
has to move from point A somewhat away from a straight line to point B to point
C--and only then to point B. In contrast, in free solution, the solute can
diffuse directly from point A to point B. This tortuosity therefore decreases
the effective concentration gradient and thus the speed at which the biological
molecule to be separated diffuses into the particle skeleton, thus reducing the
performance of the media. Values for the tortuosity parameter .tau. can be
determined by first measuring solute mass transfer rates of a very small solute
in a porous support particle and then extracting an effective

intraparticle
diffusivity from these rates, taking the support geometry and the solution-phase solute concentration driving force into proper account. Next,
one measures the void fraction .epsilon..sub.p of the porous support (e.g., by
mercury porosimetry or other standard method as discussed further below). The
tortuosity parameter .tau. is then obtained by dividing the product of the
solution-phase solute diffusivity D.sub.f (see below) times the support
particle porosity .epsilon..sub.p by the effective intraparticle solute
diffusivity. This tortuosity factor can be measured with a small diffusive
probe, or by comparing the intraparticle diffusivity of a series of variously
sized molecules. See Coffman, J. L., Ph.D. Thesis, University of Wisconsin,
1994. .tau. usually has a value of from about 2 to about 6 for many
chromatographic materials (See Coffman, J. L., Ph.D. Thesis, University of
Wisconsin, 1994). A conservative .tau. value of 2 has been assumed in E and
E* calculations presented here.

DEPR:
.epsilon..sub.p is the fractional void volume of the skeleton of the **composite**
media, which also represents the effective area open for diffusion of the
biological molecule of interest in the skeleton of the **composite**. It
represents the fraction of the volume of the support particle occupied by pores
before those pores are at least partially filled with the polymer network.
This parameter .epsilon..sub.p can be measured by mercury intrusion by those
skilled in the art.

DEPR:
The parameter .epsilon..sub.p * is the species-dependent inclusion porosity or
species-dependent void volume fraction of the **composite** media with the polymer
network present within the pores. It measures the volume fraction of the

composite particle (i.e., gel plus support particle) that is accessible to the solute and is related to the partition coefficient of the biological molecule under nonbinding conditions, that is, conditions under which the solute interacts with the polymer network or gel within the pores of the support exclusively via steric interactions. This factor, .epsilon..sub.p *, also represents the effective area open for diffusion of the biological molecule of interest in the supported gel.

DEPR:
For **composite** media, .epsilon..sub.p * derives from two effects. One is the fact that the support or skeleton of the **composite** media takes up space in the media, leaving only the void volume .epsilon..sub.p accessible to solute. Secondly, this porous volume contains gel in the **composite** media of the invention, and the polymer molecules of this gel can be arranged in such a way as to create a fine three-dimensional mesh or network. In the media of the present invention, this effective mesh size is very small, on the order of the size of biological molecules of interest. Since the mesh size is so small, many biological molecules of interest do not fit well into, and are thus at least partially excluded from, the effective pores or spaces between the polymer molecules comprising the mesh, which further and significantly reduces the solute accessible volume and thus makes .epsilon..sub.p * significantly smaller than the support void fraction .epsilon..sub.p. This steric exclusion by the polymeric network can be theoretically described by the so-called Ogston equation ##EQU7## where a is the Stokes radius of the biological molecule of interest, a.sub.f is the effective radius of a polymer strand, and .phi. is the volume fraction of the polymer forming the network. For the present invention, .phi. is the order of several percent (e.g.

preferably, 0.03 to
0.20); a.sub.f is equal to 6.5 .ANG. for polyacrylamide gels. The Stokes
radius for globular proteins can be obtained from the correlation

DEPR:
The effective intraparticle diffusivity can be measured in several ways,
including a batch uptake method, a shallow bed chromatography method, and by
analyzing breakthrough curves on a long column as discussed further below. The
parameter q.sub.0 is the intraparticle concentration of the biological
macromolecule of interest in the **composite** media at equilibrium with a
solution-phase solute concentration of C.sub.o.

DEPR:
Table I compares values of E determined for some embodiments of the present
invention (first three entries) with values of E calculated for certain prior
art **composite** media systems. Methods for the determination or estimation of
the various parameters that comprise E are discussed in more detail in the
Examples section below.

DEPR:
The unexpectedly superior mass transfer performance of the present invention is
better described by taking into account the expected steric effects of the gel
present within the pores of the **composite** medium. This is done through the
diffusional flux enhancement factor E* ##EQU8## where the effect of steric
exclusion of the solute by the gel on the expected intraparticle solute flux is
included in the species-dependent inclusion porosity .epsilon..sub.p * as
described above. E* quantitates the degree of flux enhancement through the
area that is actually open for diffusion through the supported gel, as opposed
to E, where the open area for diffusion is presumed to be the entire open area
of the skeleton or porous support. In accordance with the present invention,

E* is generally above about 3 and is preferably at least about 50 and most preferably at least about 300.

DEPR:
Table II compares values of E* determined for some embodiments of the present invention (first three entries) with values of E* calculated for certain prior art **composite** media systems.

DEPR:
The **composite** media of the present invention are superior to prior-art **composite** media by virtue of their enhanced intraparticle mass transfer rates. That is, the values of E and E* determined for the HyperD media of the present invention are typically an order of magnitude or more higher than those of prior-art supported-gel **composite** media (see Tables I and II).

DEPR:
The **composite** media of the present invention are also superior to prior-art non-**composite** media (e.g., unsupported gels, porous silica, etc.) not only by virtue of their high mass transfer efficiency but also by virtue of their improved rigidity and other mechanical properties which enable their use in high-speed chromatographic operations. Table III.A shows the values of the flux enhancement factor E computed for non-**composite** media, while Table III.B shows the calculated values of the second flux enhancement factor E* for the same prior art media.

DEPR:
Without wishing to be limited by theory, it is currently believed that the media of the present invention provide large values for E and E* compared to prior conventional media because of enhanced mobility of the biological molecules of interest inside the media. This enhanced mobility results, it is believed, from the fact that when the biological molecule of interest interacts

with and/or adsorbs or binds to the media of the present invention, the biological molecule remains appreciably mobile. Moreover, because the binding capacity q.sub.0 is high, the concentration of bound but mobile protein (or other biological on non-biological solute) can be high--leading to large and steep intraparticle concentration gradients that give rise to large intraparticle diffusional fluxes. In many conventional media, when the biological molecule of interest adsorbs or binds to the media, the biological molecule for the most part ceases to move and remains essentially stationary. This decreases the total flux of biological molecules into conventional sorbents as compared to the flux of molecules into the **composite** media described herein.

DEPR:
Under strong adsorption conditions, it may be that only one molecule out of one or several thousands of molecules will be unbound or unadsorbed in both conventional media and in the **composite** media described herein. This means that under strong binding conditions favorable for separating biological molecules, perhaps only about one of a thousand solute molecules are appreciably mobile in conventional media, the rest being bound or adsorbed to the matrix in a relatively immobile or stationary condition. Under similar binding conditions, however, a majority if not nearly all of the solute molecules within the media of the present invention are mobile, whether or not they are interacting with the polymeric network. The consequence of this is that in the media of the present invention, the driving force for intraparticle diffusion is much larger, as large as a thousand or more times larger, than is the driving force for intraparticle diffusion in conventional media. In fact, the driving force for diffusion in the media of the present

invention can approach the gradient in the total intraparticle solute concentration. The driving force for most conventional media, on the other hand, is limited to the unbound solute concentration, which is significantly smaller. Since the driving force for diffusion in the media of the present invention is so much higher, the flux is significantly greater into the present media than into conventional media. That the (biological) solute molecule of interest can remain mobile while interacting with and/or adsorbed or bound to the chromatographic media of the present invention to the extent described herein was entirely unexpected.

DEPR:
The total protein concentration in the supported-gel media, the gradient of which is the driving force for mass transfer in the present invention, is a function of the static capacity of the media for the particular solute of interest. This static capacity depends on, among other factors, the salt concentration, the pH, the properties of the gel, and the solute species. When the static sorption capacity is significantly higher than the free solution concentration, then the rate of intraparticle mass transfer in the above-mentioned **composite** particle will be largely independent of the external solution concentration. One consequence of this is that the flux enhancement factors E and E* characteristic of the invention get progressively higher as the external solution concentration decreases. This is due to the fact that the flux enhancement factors E and E* compare the rate of intraparticle diffusion within the gel-containing **composite** particle of the present invention with the rates of diffusional mass transfer within, respectively, (i) the porous support particle devoid of gel or (ii) the porous support particle

containing gel that interacts only sterically with the solute of interest.
Since the driving force decreases with decreasing external solution
concentration in the latter instances, whereas in the present media the driving
force remains relatively independent of the external solution concentration, E
and E* increase with decreasing C.sub.o. This effect makes the **composite** media
of the present invention valuable for efficiently adsorbing biological
macromolecules from dilute solutions of the biological macromolecule of
interest. Dilute solutions of biological macromolecules dominate the
biotechnology industry, as fermentation, for instance, produces relatively low
concentrations of the biological macromolecule of interest. Solute
concentrations vary widely from application to application, but C.sub.0 values
are generally at least 1 .mu.g/mL, more typically are or order 10 .mu.g/mL or
greater, and preferably are or 100 .mu.g/mL and larger. Similar considerations
apply to the use of the **composite** media in recovery of valuable metals from
dilute solutions and in the capture of environmental pollutants, and other
applications.

DEPR:
Without wishing to be limited by theory, it is believed that the high capacity
of the present **composite** media and the high mass transfer rates in that media
are aided by the flexibility of the gel polymer network incorporated into the
rigid porous support. The flexibility of the gel allows for the solute to
penetrate the gel by either the solute pushing aside the polymeric network gel
in order to get by, or by the gel molecules spontaneously moving to form a hole
into which the solute can move. This is particularly important where the
effective diameter of the solute is large in comparison with the characteristic
length between the polymer chains that form the three-dimensional

polymeric gel
network--e.g., where the solute is a biological macromolecule like a protein, a
polysaccharide, a polynucleotide or others. It is further believed that the
three-dimensional nature of the gel and the small mesh size of the gel
contribute to the high capacity of the media and to the ability of large
solutes (e.g., biological macromolecules) to move even while interacting with
the active portions, e.g., ion exchange sites or affinity sites, of the gel.
The fineness of the polymeric network mesh also means that these sites are in
close proximity, such that molecules can move from site to site quickly,
without having to desorb and/or move very far between sites.

DEPR:
Without wishing to be limited by theory, one can speculate that the confinement
of the polymeric gel network of the present invention within the pores of the
rigid porous support matrix may also be important to the operation of the
invention--in particular, to the features of high binding capacity and high
intraparticle diffusive mass transfer rates. If the polymeric gel network of
the present invention were "free" or unconfined--as opposed to being confined
within the porous volume of the support matrix of the present **composite** media,
then the as polymerized gel swells or increases in volume several-fold when
exposed to dilute aqueous solutions of the sort normally encountered in
biochromatography; this swelling results from, e.g., in ion-exchange gel media
or in affinity media where the affinity to the biological macromolecule of
interest is to some extent ionic (such as heparin affinity or lysine affinity
for example), the repulsion of fixed charges of like sign on the polymeric
network. This swelling effectively "dilutes" the binding sites (thus reducing
binding capacity), and, under certain circumstances, may make it necessary for

a solute to disassociate from one binding site before diffusing to and interacting with another. In contrast, with the confined gel of the present invention, the polymeric network cannot swell, despite the strong interaction of the fixed ionic charges. As a consequence, binding capacity remains high. Moreover, the regions of ionic interaction overlap, and a solute of opposite charge (such as a biological macromolecule) can move freely within the entire polymer network while interacting electrostatically with more than one ionic group or affinity site of the three dimensional polymeric network. That is, a sorbed solute molecule may not have to dissociate from one binding "site" before diffusing to and interacting with another, since the binding "sites" are not necessarily discrete in the polymeric network contained within the **composite** media of the present invention. This has clearly unexpected advantages in terms of enhanced intraparticle mass transfer rates.

DEPR:
In a preferred embodiment, the present invention provides a passivated **composite** sorbent particle comprising a porous solid matrix having interior and exterior surfaces and innate (i.e., inherently present) groups that render the matrix susceptible to undesirable non-specific interaction with biological molecules, and a polymer network derived from a passivation mixture comprising effective amounts of a main monomer, a passivating monomer different from the main monomer, and a crosslinking agent, the mixture having been allowed to come into intimate contact with the surfaces of the matrix for a sufficient period of time such that on polymerization of the mixture the innate groups of the matrix become deactivated, resulting in the minimization or substantial elimination of the above-mentioned undesirable non-specific interactions.

DEPR:
The passivated **composite** media of the present invention are further characterized by reversible high sorptive capacity and high intraparticle diffusive mass transfer rates for biological molecules including proteins. Furthermore, the passivated **composite** media of the present invention enjoy exceptional chemical stability on exposure to strongly acidic or alkaline media and/or strong oxidizing solutions such as those that are frequently utilized during cleaning of industrial manufacturing equipment.

DEPR:
While it has been discovered that this process of depositing protective polymer coatings upon the porous surfaces of mineral oxide (and particularly silica) matrices can significantly stabilize these materials by sharply reducing their rates of chemical leaching, the approach has the important disadvantage of rendering the porous surfaces of the coated and protected matrices hydrophobic and thus prone to cause excessive non-specific binding of proteins by adsorption. (This is precisely the same problem noted above in connection with entirely polymeric porous support matrices.) However, this problem can be successfully addressed by the methods of the present invention in the same way as the non-specific binding of strictly polymeric support matrices can be reduced --i.e., by passivation in a process of oriented polymerization. More particularly, these **composite** chromatographic media (i.e., supports comprised of mineral oxide substrates that have been stabilized by the application of thin protective polymer coatings) can be passivated against excessive non-specific binding by incorporating passivating ("neutralizing") monomers capable of associating with and consequently deactivating innate non-polar hydrophobic groups exposed on the matrix surface. The

passivating monomers
useful in this embodiment of the present invention adsorb upon (and
consequently cover) the hydrophobic groups on the surface by virtue of their
containing long-chain saturated hydrocarbons, olefinic hydrocarbon groups,
aromatic groups, or like hydrophobic domains that interact with and become
appreciably bound to their hydrophobic counterparts on the matrix surface as a
consequence of the hydrophobic-hydrophobic interaction existing between them.

DEPR:
The size exclusion limit of the **composite** media varies somewhat but generally
falls in the range of about 500 to about 2,000,000 Daltons, preferably, 50,000
to about 500,000. The sorptive capacity can also be manipulated, depending on
the amount of main monomer incorporated in the polymer network, and ranges
between about 1 milligram to about 300 milligrams of solute or biological
molecule per unit volume (mL) of media--preferably at least about 50 mg/mL, and
most preferably about 100 mg/mL.

DEPR:
Eluent solutions suitable for use in the present invention are well known to
those of ordinary skill in the art. For example, a change in ionic strength,
pH or solvent composition may be effective in "stepwise" elution processes.
Alternately, eluent solutions may comprise a salt gradient, a pH gradient or
any particular solvent or solvent mixture that is specifically useful in
displacing the preselected biological molecule. Such methods are generally
known to those engaged in the practice of protein chromatography. Still
another object of the present invention relates to a chromatographic method for
the separation of biological molecules comprising passing a sample containing a
mixture of biological molecules through a column packed with the
composite

media disclosed herein.

DEPR:
By virtue of their superior mass transfer characteristics and high binding capacity, it is anticipated that the **composite** particles of the present invention will also find use outside the field of biochromatography--both as adsorbents (in chromatographic and other types of sorption processes, e.g., for the recovery of environmental pollutants, valuable metals, etc.) and as solid-phase supports for the conduct of chemical reactions, the immobilization of reactants and catalysts, and the capture of reaction products.

DEPR:
In another embodiment, the polymerization step can take place in the presence of a pore inducer. The pore inducers of the present invention allow polymerization of the gel to take place while maximizing the accessibility of the interior volume of the **composite** media. Suitable pore inducers, also referred to as porogens, used in the present invention include, but are not limited to, polyethylene glycols, polyoxyethylenes, polysaccharides such as dextran, and polar solvents. Polar solvents include those commonly used in chemical synthesis or polymer chemistry and known to those skilled in the art. Suitable polar solvents include alcohols, ketones, tetrahydrofuran, dimethylformamide, and dimethysulfoxide. Preferred polar solvents are ethanol, methanol, dioxane, and dimethysulfoxide.

DEPR:
In all cases, i.e., whether the porous matrix is comprised of a mineral oxide, a polymer-coated and thus stabilized mineral oxide, or a polymer, the polymerization process of the present invention creates a three-dimensional lattice or crosslinked polymer network that extends away from the pore-wall surfaces of the porous solid matrix. Again, not wishing to be

limited by theory, it is believed that this polymer network is comprised of a thin passivating region or layer that ideally interacts with the surface of any non-specific adsorption sites of the solid support (e.g., silanols in the case of silica) covalently linked with a three-dimensional structural polymer lattice that can (but need not necessarily) substantially fill the porous volume. In a preferred embodiment, the three-dimensional shape of the polymer lattice is believed to be substantially identical to the shape of the pore which it fills (see FIG. 5), with any passivating layer oriented adjacent to and continuous (i.e., covalently linked) to the three-dimensional polymer lattice that extends away from the matrix surface. This configuration prevents "neutralizing" or "deactivating" pieces of the polymer network from eluting from the **composite** media during regular use--for example, when it is exposed to vigorous washing or cleaning conditions, such as high acidic pH, high alkaline pH, high ionic strength, and strong oxidizing conditions. This crosslinked polymer network creates a novel chromatographic sorbent which can then be used, for example, in a process for separating and purifying various biomolecules, including macromolecules.

DEPR:
Indeed, it has been surprisingly discovered that the **composite** media of the present invention manifest chromatographic characteristics that are unparalleled under several criteria, particularly in terms of dynamic sorptive capacity as a function of flow rate and high intraparticle mass transfer rates. In particular, whereas the great majority of porous materials suffer a marked decrease in useful sorptive capacity as flow rates increase (e.g., at flow rates of about 50 cm/hr or greater), the passivated porous supports of the

present invention show little decrease in useful sorptive capacity from a static condition up to flow rates approaching several hundred centimeters per hour. Compare, for example, the behavior of prior art "gel"-type materials with the supports of the present invention, as illustrated in the graphs of FIG. 3A, 3B, and 4 (described further in Example 16).

DEPR:
Moreover, the absolute capacities of the **composite** media of the present invention are considerably greater than those attained with other types of chromatographic media (e.g., Spherodex.TM.) Thus, as shown in FIG. 4, a plot of the absolute capacity vs. flow rate of various chromatographic media unambiguously shows that the **composite** media of the present invention combines a very high absolute sorption capacity (expressed as mg/mL) with a relative insensitivity to solution flow rates.

DEPR:
It is believed, without wishing to be limited by theory, that a flexible lattice structure comprised primarily of polymeric chains of repeating main monomer units is formed within the pores of the porous solid matrix. Very significantly, it is believed that the areas of the **composite** media available for desirable reversible interaction with biological molecules are not confined to the regions immediately adjacent to the surface of the pore as is the case when thin, substantially two-dimensional coatings are applied to porous surfaces in the manner of Steuck (U.S. Pat. No. 4,618,533) and Varady et al. (U.S. Pat. No. 5,030,352) as discussed in the Background of the Invention section above. Rather, it is believed that the gel-type polymeric network of the present invention extends outwardly into the pore volume itself in the manner of a three-dimensional preferably but not necessarily pore-filling

lattice, as opposed to a two-dimensional coating limited strictly to the pore wall surface area. A schematic diagram of such a structure, as it is thought to exist, is illustrated in FIG. 5, where a biological molecule of interest (depicted as a spherical object) is also shown interacting with the lattice. Furthermore, the presence of porogens (pore-inducers) in the passivation mixture is believed to promote creation of this three-dimensional polymer network.

DEPR:
It is further thought that such an extended polymer network contributes not only to the unusually high absolute sorptive capacity of the composite media of the invention as measured under static (i.e., no flow) conditions, but also permits rapid intraparticle mass transfer by diffusion and thereby allows the present invention to maintain high sorptive capacities largely independent of solution flow rates. It is thought that perhaps the flexible nature of the three-dimensional polymer network allows biological molecules to rapidly penetrate the polymer lattice and thereby efficiently interact with sorptive groups in the polymer network of the passivated porous support while maintaining their mobility even at high solution flow rates. The rapid and efficient mass transfer of biomolecules into and through this network avoids the decrease in useful or dynamic sorption capacity and resolution that are typical of conventional chromatographic media. With these conventional media, diffusion in the pores of the particle and/or materials coated thereupon or within them can be slow, leading to poor mass transfer rates and poor efficiency of the chromatographic process.

DEPR:
Thus, a method of performing chromatographic separations characterized by high

sustained sorptive capacity relatively independent of flow rate and rapid,
efficient intraparticle mass transfer is achieved with **composite** media of the
present invention, which media include a flexible three-dimensional network or
lattice of crosslinked polymer chains extending within and throughout the pores
of the support matrix.

DEPR:
The separation and purification process usually involves at least two steps.
The first step is to charge a packed or fluidized bed column containing the
preferably passivated **composite** adsorbent with a solution containing a mixture
of biomolecules, at least one of which it is desired to separate and recover in
at least partially purified form. The second step is to pass an eluent
solution through the column to effect the release of the biomolecules from the
column, thereby causing their separation.

DEPR:
It is thus demonstrated that the level of non-specific adsorption for lysozyme
(a strong cationic protein) is high when the MAPTAC is absent. The
non-specific adsorption for silica with large surface are (X 075, 100 m.sup.2
/g) is higher (55 mg/mL of **resin) than the non-specific adsorption for silica** X
015 (25 m.sup.2 /g; 15 mg/mL of resin). A certain proportionality exists
between the surface area and the original level of non-specific absorptions.
The amount of MAPTAC to decrease the level of non-specific adsorption down to
zero is also proportional to the surface area available: 1.5% of MAPTAC is
necessary with silica X 015 (25 m.sup.2 /g) whereas at least 6% is necessary to
passivate silica X 075 (100 m.sup.2 /g).

DEPR:
The passivated resin is then recovered by filtration. The oil is eliminated
with an extensive washing with water containing 0.1-0.5% of a

non-ionic
detergent and then stored in a saline buffer at neutral pH. The product resin
shows very similar ion-exchange characteristics as those described in Example
2. Additionally, its sensitivity in strong alkaline media is much improved as
measured by its weight loss after one night of contact with 0.5M sodium
hydroxide. The passivated resin of this example lost only about half as much
weight as an anionic **resin prepared from silica** having an unprotected surface
area.

DEPR:
Various polyacrylamide/silica ~~composites~~ prepared as described hereinabove (and
according to U.S. Pat. No. 5,268,097) are used as supports in accordance ~~with~~
the present invention in the Examples which follows. These materials are
referred to hereinafter as S-HyperD F, S-HyperD M, Q-HyperD F, and Q-HyperD M.
The S-HyperD media is a series of cation exchange chromatography media of
various particle sizes. The Q-HyperD series is a series of anion exchange
chromatography media of various particle sizes. The "F" and "M" suffixes refer
to HyperD media particle sizes, with F indicating nominal media particle
diameters of 35 micrometers and M indicating nominal media particle diameters
of 60 micrometers.

DEPR:
Two different polyacrylamide/silica **composites** were prepared as described above
and are referred to hereinafter as Q-HyperD F and Q-HyperD M. The water
contents of the Q-HyperD F and M media samples were determined. The particle
density of hydrated Q-HyperD media was measured to be 1.424 g/cm. The particle
sizes and particle size distributions were measured optically.

DEPR:
A polyacrylamide/silica **composite** was prepared as described above and is

referred to hereinafter as Q-HyperD M. The water content of the Q-HyperD M
media samples was determined. The particle density of hydrated Q-HyperD M
media was calculated to be 1.424 g/cm.sup.3. The particle size and particle
size distribution were measured optically.

DEPR:
A polyacrylamide/silica **composite** was prepared as described above and is
referred to hereinafter as Q-HyperD M. The water content of the Q-HyperD M
media samples was determined. The particle density of hydrated Q-HyperD M
media was calculated to be 1.424 g/cm.sup.3. The particle size and particle
size distribution were measured optically.

DEPR:
Polyacrylamide/silica **composites** were prepared as described above and are
referred to hereinafter as Q-HyperD F and as Q-HyperD M.

DEPR:
Polyacrylamide/silica **composites** were prepared as described above and are
referred to hereinafter as Q-HyperD F and as Q-HyperD M.

DEPR:
Polyacrylamide/silica **composites** were prepared as described above and are
referred to hereinafter as Q-HyperD F and as Q-HyperD M.

DEPR:
Substitution of these and other parameters in the above expression yields bed
void volumes .epsilon..sub.b of 0.5, 0.48, 0.49, and 0.5 for four columns
packed with the HyperD **composite** media of the present invention.

DEPR:
A polyacrylamide/silica **composite** was prepared as described above and is
referred to hereinafter as Q-HyperD F.

DEPR:
The species-dependent void volume of the media in the present invention can be
adequately predicted by applying the Ogston equation to describe

exclusion by
the polymer network of the **composite** media--modifying the Ogston equation to
take into account the volume taken up by the skeleton or porous support
particle that forms the base matrix for the **composite** media of this invention.
The Ogston equation so modified is shown below: ##EQU16## where a is the Stokes
radius of the biological molecule of interest, a.sub.f is the effective radius
of a strand of polymer forming the gel network, and .phi. is the volume
fraction of the polymer forming the network. For the HyperD embodiment of
present invention based on a silica-supported polyacrylamide gel, the polymer
volume fraction .phi. is equal to 0.105, and the effective radius of a strand
of polyacrylamide is 6.5 .ANG.. The Stokes radius for globular proteins can be
obtained from the correlation

DEPL:
The **composite** media

DEPL:
Preparation of an Anion-Exchange **Resin Using a Surface-Protected (i.e., Pre-coated) Silica** Passivated Porous Support

DEPL:
Determination of Ion-Exchange and Protein Sorption Capacity of Preparation of
Anion-Exchange **Resins Based on Passivated Porous Silica** Support of Different
Surface Areas

DEPL:
Determination of adsorption capacity (BSA on polyacrylamide/silica **composites**)

DEPL:
Determination of adsorption capacity (ovalbumin on polyacrylamide/silica
composite)

DEPL:
Determination of adsorption capacity (.alpha.-lactalbumin on polyacrylamide/silica **composite**)

DEPL:
From the agreement between .epsilon..sub.p * values for Dextran T-40 and BSA,
it can be seen that .epsilon..sub.p * is independent of salt concentration
between salt concentrations up to at least 1000 millimolar salt. It can also
be seen from .epsilon..sub.p * values for blue dextran (a very large solute,
having a molecular weight of approximately 2,000,000) and BSA that molecules as
large or larger than BSA have .epsilon..sub.p * values less than about 0.01.
The fact that these .epsilon..sub.p * values are generally much smaller than
unity illustrates that, under nonbinding/noninteractive conditions, the degree
of steric exclusion of solute by the supported polymeric gel network of the
present invention is indeed appreciable. Given this steric effect, it is thus
particularly unexpected that the rates of intraparticle diffusive mass transfer
can be as large as observed--as exemplified by the large values of the flux
enhancement factor E* calculated for **composite** media of the present invention.

CLPR:
2. The method of claim 1, wherein said **composite** media provides a larger
dynamic capacity for said macromolecule at initial feed concentrations of less
than about 2 milligrams per milliliter than the dynamic capacity provided by
the same media for the same macromolecule at initial feed concentrations of
greater than about 2 milligrams per milliliter.

CLPR:
6. The method of claim 1, which further comprises selecting the **composite**
media to be a chromatographic media so that said biological macromolecules can
be separated by chromatography.

CLPV:
selecting a **composite** media comprising a porous support comprising voids

containing a polymeric network, wherein the **composite** media has a size
exclusion limit of about 500 Daltons to about 2,000,000 Daltons and provides a
value of a flux enhancement factor E greater than about 3, as determined by the
equation ##EQU21## in which D.sub.s is the experimentally measured effective
intraparticle diffusivity of a biological molecule of interest,

CLPV:
.epsilon..sub.p is the fractional void volume of the porous support of the
composite media,

CLPV:
.tau. is the tortuosity of the porous support of the **composite** media, and

CLPW:
passing, at a flow rate of at least about 50 cm/hr, a sample containing a
mixture of biological macromolecules including a biological macromolecule of
interest, wherein initial C.sub.0 is less than about 2 milligrams per
milliliter, through a column packed with the **composite** media which provides a
larger dynamic capacity for said biological macromolecule of interest at a low
concentration than the dynamic capacity provided by the same media for the same
macromolecule at a higher concentration; and

CCXR:
210/198.2

DOCUMENT-IDENTIFIER: US 5738783 A
TITLE: Liquid chromatograph having a micro- and semi-micro column

DEPR:
In the present invention, porous media commonly used in liquid chromatography
such as silica gel, alumina, porous glass beads, zeolite, hydroxyapatite and
graphite may be used as the packing material of the column 40. Alternatively,
it is possible to use **composite** powders formed of a **resin core covered by**
inorganic powders such as silica gel, titanium dioxide, hydroxyapatite, and the
like. On may use materials such as polyamides, acrylic resins, polyvinyl
alcohols, and the like, for the resin core.

CCOR:
210/198.2

DOCUMENT-IDENTIFIER: US 5672276 A
TITLE: Passivated porous polymer supports and methods for the preparation and
use of same

BSPR:
Several previous investigators have sought to passivate various microporous
media including membranes and particulate chromatographic supports by applying
thin surface coatings to inorganic or organic/polymeric substrates. For
example, Steuck, in U.S. Pat. No. 4,618,533, discloses a porous polymeric
membrane substrate fashioned from a thermoplastic organic polymer upon which a
permanent coating is grafted and/or deposited on the entire membrane surface.
The polymerization and crosslinking of the polymerizable monomer upon and
within the porous membrane substrate is performed in such a way that a thin
coating is deposited upon the entire surface of the porous membrane, including
the inner pore walls. Significantly, the porous configurations of the coated,
composite membrane structures claimed by Steuck are essentially identical to
those of the corresponding uncoated porous membrane substrates, implying that
the polymer of Steuck is applied as a thin surface layer or coating that does
not interfere with the porosity or flow properties of his **composite** membranes.
Moreover, Steuck does not disclose the concept of a "passivating layer" or the
use of monomers capable of functioning as "passivating" monomers within the
meaning of the present invention as discussed in more detail below.

BSPR:
While it has been discovered that this process of depositing protective polymer
coatings upon the porous surfaces of mineral oxide (and particularly silica)
matrices can significantly stabilize these materials by sharply reducing their

rates of chemical leaching, the approach has the important disadvantage of rendering the porous surfaces of the coated and protected matrices hydrophobic and thus prone to cause excessive non-specific binding of proteins by adsorption. (This is precisely the same problem noted above in connection with entirely polymeric porous support matrices.) However, this problem can be successfully addressed by the methods of the present invention in the same way as the non-specific binding of strictly polymeric support matrices can be reduced--i.e., by passivation in a process of oriented polymerization. More particularly, these **composite** chromatographic supports (i.e., supports comprised of mineral oxide substrates that have been stabilized by the application of thin protective polymer coatings) can be passivated against excessive non-specific binding by incorporating passivating ("neutralizing") monomers capable of associating with and consequently deactivating innate non-polar hydrophobic groups exposed on the matrix surface. The passivating monomers useful in this embodiment of the present invention adsorb upon (and consequently cover) the hydrophobic groups on the surface by virtue of their containing long-chain saturated hydrocarbons, olefinic hydrocarbon groups, aromatic groups, or like hydrophobic domains that interact with and become appreciably bound to their hydrophobic counterparts on the matrix surface as a consequence of the hydrophobic-hydrophobic interaction existing between them.

Typically, the present invention utilizes base matrices having the following characteristics: an initial average particle size ranging from about 5 to about 1000 microns; an initial porous volume ranging from about 0.2 to about 2 cm.sup.3 /gram; an initial surface area ranging from about 1 to about 800 m.sup.2 /gram; and an initial pore size ranging from about 50 to about 6000

angstroms. Preferably, the base matrix is characterized by: an initial average particle size ranging from about 10 to about 300 microns, although passivated supports having narrow particle size ranges, such as about 15-20, about 15-25, about 30-45, about 50-60, about 80-100, and about 100-300 microns, are most preferred. Preferred ranges for other characteristics include an-initial porous volume ranging from about 0.8 to about 1.2 cm.sup.3 /gram; an initial surface area ranging from about 10 to about 400 m.sup.2 /gram; and an initial pore size ranging from about 1000 to about 3000 angstroms. The density of the porous solid matrix obviously varies with its chemical nature, being higher for mineral oxide (e.g., silica) substrates and lower for polymeric ones (e.g., polystyrene).

DEPR:
It is thus demonstrated that the level of non-specific adsorption for lysozyme (a strong cationic protein) is high when the MAPTAC is absent. The non-specific adsorption for silica with large surface ares (X 075, 100m.sup.2 /g) is higher (55 mg/ml of **resin) than the non-specific adsorption for silica** X 015 (25 m.sup.2 /g; 15 mg/ml of resin). A certain proportionality exists between the surface area and the original level of non-specific absorptions. The amount of MAPTAC to decrease the level of-non-specific adsorption down to zero is also proportional to the surface area available: 1.5% of MAPTAC is necessary with silica X 015 (25 m.sup.2 /g) whereas at least 6% is necessary to passivate silica X 075 (100 m.sup.2 /g).

DEPR:
The passivated resin is then recovered by filtration. The oil is eliminated with an extensive washing with water containing 0.1-0.5% of a non-ionic detergent and then stored in a saline buffer at neutral pH. The product resin

shows very similar ion-exchange characteristics as those described in Example 2. Additionally, its sensitivity in strong alkaline media is much improved as measured by its weight loss after one night of contact with 0.5M sodium hydroxide. The passivated resin of this example lost only about half as much weight as an artionic **resin prepared from silica** having an unprotected surface area.

DEPL:
Preparation of an Anion-Exchange **Resin Using a Surface-Protected (i.e., Pre-coated) Silica** Passivated Porous Support

DEPL:
Determination of Ion-Exchange and Protein Sorption Capacity of Preparation of Anion-Exchange **Resins Based on Passivated Porous Silica** Support of Different Surface Areas

CCXR:
210/198.2

DOCUMENT-IDENTIFIER: US 5599453 A
TITLE: Passivated porous supports and methods for the preparation and use of
same

BSPR:
Several previous investigators have sought to passivate various microporous
media including membranes and particulate chromatographic supports by applying
thin surface coatings to inorganic or organic/polymeric substrates. For
example, Steuck, in U.S. Pat. No. 4,618,533, discloses a porous polymeric
membrane substrate fashioned from a thermoplastic organic polymer upon which a
permanent coating is grafted and/or deposited on the entire membrane surface.
The polymerization and crosslinking of the polymerizable monomer upon and
within the porous membrane substrate is performed in such a way that a thin
coating is deposited upon the entire surface of the porous membrane, including
the inner pore walls. Significantly, the porous configurations of the coated,
composite membrane structures claimed by Steuck are essentially identical to
those of the corresponding uncoated porous membrane substrates, implying that
the polymer of Steuck is applied as a thin surface layer or coating that does
not interfere with the porosity or flow properties of his **composite** membranes.
Moreover, Steuck does not disclose the concept of a "passivating layer" or the
use of monomers capable of functioning as "passivating" monomers within the
meaning of the present invention as discussed in more detail below.

BSPR:
While it has been discovered that this process of depositing protective polymer
coatings upon the porous surfaces of mineral oxide (and particularly silica)
matrices can significantly stabilize these materials by sharply reducing their

rates of chemical leaching, the approach has the important disadvantage of rendering the porous surfaces of the coated and protected matrices hydrophobic and thus prone to cause excessive non-specific binding of proteins by adsorption. (This is precisely the same problem noted above in connection with entirely polymeric porous support matrices.) However, this problem can be successfully addressed by the methods of the present invention in the same way as the non-specific binding of strictly polymeric support matrices can be reduced--i.e., by passivation in a process of oriented polymerization. More particularly, these **composite** chromatographic supports (i.e., supports comprised of mineral oxide substrates that have been stabilized by the application of thin protective polymer coatings) can be passivated against excessive non-specific binding by incorporating passivating ("neutralizing") monomers capable of associating with and consequently deactivating innate non-polar hydrophobic groups exposed on the matrix surface. The passivating monomers useful in this embodiment of the present invention adsorb upon (and consequently cover) the hydrophobic groups on the surface by virtue of their containing long-chain saturated hydrocarbons, olefinic hydrocarbon groups, aromatic groups, or like hydrophobic domains that interact with and become appreciably bound to their hydrophobic counterparts on the matrix surface as a consequence of the hydrophobic-hydrophobic interaction existing between them.

Typically, the present invention utilizes base matrices having the following characteristics: an initial average particle size ranging from about 5 to about 1000 microns; an initial porous volume ranging from about 0.2 to about 2 cm.sup.3 /gram; an initial surface area ranging from about 1 to about 800 m.sup.2 /gram; and an initial pore size ranging from about 50 to about 6000

angstroms. Preferably, the base matrix is characterized by: an initial average particle size ranging from about 10 to about 300 microns, although passivated supports having narrow particle size ranges, such as about 15-20, about 15-25, about 30-45, about 50-6, about 80-100, and about 100-300microns, are most preferred. Preferred ranges for other characteristics include an initial porous volume ranging from about 0.8 to about 1.2 cm.sup.3 /gram; an initial surface area ranging from about 10 to about 400 m.sup.2 /gram; and an initial pore size ranging from about 1000 to about 3000 angstroms. The density of the porous solid matrix obviously varies with its chemical nature, being higher for mineral oxide (e.g., silica) substrates and lower for polymeric ones (e.g., polystyrene).

DEPR:
It is thus demonstrated that the level of non-specific adsorption for lysozyme (a strong cationic protein) is high when the MAPTAC is absent. The non-specific adsorption for silica with large surface ares (X 075, 100 m.sup.2 /g) is higher (55 mg/ml of **resin) than the non-specific adsorption for silica** X 015 (25 m.sup.2 /g; 15 mg/ml of resin). A certain proportionality exists between the surface area and the original level of non-specific absorptions. The amount of MAPTAC to decrease the level of non-specific adsorption down to zero is also proportional to the surface area available: 1.5% of MAPTAC is necessary with silica X 015 (25 m.sup.2 /g) whereas at least 6% is necessary to passivate silica X 075 (100 m.sup.2 /g).

DEPR:
The passivated resin is then recovered by filtration. The oil is eliminated with an extensive washing with water containing 0.1-0.5% of a non-ionic detergent and then stored in a saline buffer at neutral pH. The product resin

shows very similar ion-exchange characteristics as those described in Example 2. Additionally, its sensitivity in strong alkaline media is much improved as measured by its weight loss after one night of contact with 0.5M sodium hydroxide. The passivated resin of this example lost only about half as much weight as an anionic **resin prepared from silica** having an unprotected surface area.

DEPL:
Preparation of an Anion-Exchange **Resin Using a Surface-Protected (i.e., Pre-coated) Silica** Passivated Porous Support

DEPL:
Determination of Ion-Exchange and Protein Sorption Capacity of Preparation of Anion-Exchange **Resins Based on Passivated Porous Silica** Support of Different Surface Areas

CCXR:
210/198.2

DOCUMENT-IDENTIFIER: US 5593576 A
TITLE: Passivated porous polymer supports and methods for the preparation and
use of same

BSPR:
Several previous investigators have sought to passivate various microporous
media including membranes and particulate chromatographic supports by applying
thin surface coatings to inorganic or organic/polymeric substrates. For
example, Steuck, in U.S. Pat. No. 4,618,533, discloses a porous polymeric
membrane substrate fashioned from a thermoplastic organic polymer upon which a
permanent coating is grafted and/or deposited on the entire membrane surface.
The polymerization and crosslinking of the polymerizable monomer upon and
within the porous membrane substrate is performed in such a way that a thin
coating is deposited upon the entire surface of the porous membrane, including
the inner pore walls. Significantly, the porous configurations of the coated,
composite membrane structures claimed by Steuck are essentially identical to
those of the corresponding uncoated porous membrane substrates, implying that
the polymer of Steuck is applied as a thin surface layer or coating that does
not interfere with the porosity or flow properties of his **composite** membranes.
Moreover, Steuck does not disclose the concept of a "passivating layer" or the
use of monomers capable of functioning as "passivating" monomers within the
meaning of the present invention as discussed in more detail below.

BSPR:
While it has been discovered that this process of depositing protective polymer
coatings upon the porous surfaces of mineral oxide (and particularly silica)
matrices can significantly stabilize these materials by sharply reducing their

rates of chemical leaching, the approach has the important disadvantage of rendering the porous surfaces of the coated and protected matrices hydrophobic and thus prone to cause excessive non-specific binding of proteins by adsorption. (This is precisely the same problem noted above in connection with entirely polymeric porous support matrices.) However, this problem can be successfully addressed by the methods of the present invention in the same way as the non-specific binding of strictly polymeric support matrices can be reduced--i.e., by passivation in a process of oriented polymerization. More particularly, these **composite** chromatographic supports (i.e., supports comprised of mineral oxide substrates that have been stabilized by the application of thin protective polymer coatings) can be passivated against excessive non-specific binding by incorporating passivating ("neutralizing") monomers capable of associating with and consequently deactivating innate nonpolar hydrophobic groups exposed on the matrix surface. The passivating monomers useful in this embodiment of the present invention adsorb upon (and consequently cover) the hydrophobic groups on the surface by virtue of their containing long-chain saturated hydrocarbons, olefinic hydrocarbon groups, aromatic groups, or like hydrophobic domains that interact with and become appreciably bound to their hydrophobic counterparts on the matrix surface as a consequence of the hydrophobic-hydrophobic interaction existing between them.

Typically, the present invention utilizes base matrices having the following characteristics: an initial average particle size ranging from about 5 to about 1000 microns; an initial porous volume ranging from about 0.2 to about 2 cm.sup.3 /gram; an initial surface area ranging from about 1 to about 800 m.sup.2 /gram; and an initial pore size ranging from about 50 to about 6000

angstroms. Preferably, the base matrix is characterized by: an initial average particle size ranging from about 10 to about 300 microns, although passivated supports having narrow particle size ranges, such as about 15-20, about 15-25, about 30-45, about 50-60, about 80-100, and about 100-300 microns, are most preferred. Preferred ranges for other characteristics include an initial porous volume ranging from about 0.8 to about 1.2 cm.sup.3 /gram; an initial surface area ranging from about 10 to about 400 m.sup.2 /gram; and an initial pore size ranging from about 1000 to about 3000 angstroms. The density of the porous solid matrix obviously varies with its chemical nature, being higher for mineral oxide (e.g., silica) substrates and lower for polymeric ones (e.g., polystyrene).

DEPR:
It is thus demonstrated that the level of non-specific adsorption for lysozyme (a strong cationic protein) is high when the MAPTAC is absent. The non-specific adsorption for silica with large surface areas (X 075, 100 m.sup.2 /g) is higher (55 mg/ml of **resin) than the non-specific adsorption for silica** X 015 (25 m.sup.2 /g; 15 mg/ml of resin). A certain proportionality exists between the surface area and the original level of non-specific absorptions. The amount of MAPTAC to decrease the level of non-specific adsorption down to zero is also proportional to the surface area available: 1.5% of MAPTAC is necessary with silica X 015 (25 m.sup.2 /g) whereas at least 6% is necessary to passivate silica X 075 (100 m.sup.2 /g).

DEPR:
The passivated resin is then recovered by filtration. The oil is eliminated with an extensive washing with water containing 0.1-0.5% of a non-ionic detergent and then stored in a saline buffer at neutral pH. The product resin

shows very similar ion-exchange characteristics as those described in Example 2. Additionally, its sensitivity in strong alkaline media is much improved as measured by its weight loss after one night of contact with 0.5M sodium hydroxide. The passivated resin of this example lost only about half as much weight as an artionic **resin prepared from silica** having an unprotected surface area.

DEPC:
Preparation of an Anion-Exchange **Resin Using a Surface-Protected (i.e., Pre-coated) Silica** Passivated Porous Support

DEPC:
Determination of Ion-Exchange and Protein Sorption Capacity of Preparation of Anion-Exchange **Resins Based on Passivated Porous Silica** Support of Different Surface Areas

CCOR:
210/198.2

DOCUMENT-IDENTIFIER: US 5545317 A
TITLE: Liquid column packing materials and method for making the same

BSPR:
Liquid column (LC) packing materials are usually porous materials which possess adsorptive or catalytic sites on the pore walls. They may be used as packings in columns or as loose material in vessels. LC packing materials typically are porous particles. However, they may instead be fibers or membranes. Porous membranes also provide filtration. When the pore size of the adsorptive or catalytic membrane excludes large proteins, then ultrafiltration is combined with adsorption or catalysis. Membranes may have small pores throughout their mass which exclude protein. Alternatively, the membrane may be a **composite** of sintered or adhered porous particles, in which case the pores between particles are large whereas the pores within the sintered or adhered particles are small.
For example, Kontes Glass Co. markets a thick porous membrane of polyvinyl chloride upon whose large pore walls are attached small porous silica particles. The pores of the silica particles are much smaller than the pores upon whose walls the silica particles are attached.

BSPR:
A second approach to imparting a crosslinked protein coating onto packing materials employs simultaneous contact of glutaraldehyde with a concentrated solution of protein in an unbonded silica slurry in water. Such coated supports have high immobilized protein context and are useful for chromatography of dissolved protein. The object of this approach is to maximize the amount of immobilized protein short of creating an impermeable **composite** through which liquid could not readily flow. In this approach, the weak adsorption properties of the immobilized protein in the

packing material
are useful. See, e.g., M. Tsuboi et al, "Chromatography Carrier", Japanese
Patent Application No. 198,334/85, Sept. 7, 1985. A similar method uses a
two-stage glutaraldehyde crosslinking procedure in which the crosslinking was
interrupted after a period of time by washing away serum albumin that had not
yet deposited on the silica. Subsequently, more glutaraldehyde was added to
ensure that the remaining albumin was tightly crosslinked and permanently
attached to the silica. The two stage process ensured that large clumps of
support particles were not glued together. Such clumps disrupt flow through
the column and degrade efficiency. See, e.g., R. A. Thompson et al, "Sorbents
Obtained by Entrapment of Crosslinked Bovine Serum Albumin in Silica", Journal
Chromatography, Vol. 465 (1989) pp. 263-270.

BSPR:
Reverse phase, cation exchange, and anion exchange organic **resin or
silica**-based supports are sufficiently protein-adsorptive to be used, as is
untreated silica. The preferred porous protein-adsorptive support is a porous
silica support such as porous silica having a pore diameter of 30 to 300.ANG.,
and a particle size of 1 to 500 micrometers; although, any protein-adsorptive
support may be used. When a porous silica support is used it may be one
already having an alkylsilane bonded to the surfaces thereof or it may be a
dual zone or mixed phase material such as that shown in my U.S. Pat. No.
4,773,994, 4,778,600, 4,782,040, 4,950,634 and 4,950,635. In all instances,
the coating of crosslinked protein covers the external surfaces of the porous
silica support overcoating the alkyl or ketal-blocked-diol or fluoroalkyl or
other external phase of the dual zone or mixed phase material.

CCOR:
210/198.2

DOCUMENT-IDENTIFIER: US 5512169 A
TITLE: Liquid column packing materials

BSPR:
Liquid column (LC) packing materials are usually porous materials which possess adsorptive or catalytic sites on the pore walls. They may be used as packings in columns or as loose material in vessels. LC packing materials typically are porous particles. However, they may instead be fibers or membranes. Porous membranes also provide filtration. When the pore size of the adsorptive or catalytic membrane excludes large proteins, then ultrafiltration is combined with adsorption or catalysis. Membranes may have small pores throughout their mass which exclude protein. Alternatively, the membrane may be a **composite** of sintered or adhered porous particles, in which case the pores between particles are large whereas the pores within the sintered or adhered particles are small.
For example, Kontes Glass Co. markets a thick porous membrane of polyvinyl chloride upon whose large pore walls are attached small porous silica particles. The pores of the silica particles are much smaller than the pores upon whose walls the silica particles are attached.

BSPR:
A second approach to imparting a crosslinked protein coating onto packing materials employs simultaneous contact of glutaraldehyde with a concentrated solution of protein in an unbonded silica slurry in water. Such coated supports have high immobilized protein context and are useful for chromatography of dissolved protein. The object of this approach is to maximize the amount of immobilized protein short of creating an impermeable **composite** through which liquid could not readily flow. In this approach, the weak adsorption properties of the immobilized protein in the packing material

are useful. See, e.g., M. Tsuboi et al, "Chromatography Carrier", Japanese Patent Application No. 198,334/85, Sep. 7, 1985. A similar method uses a two-stage glutaraldehyde crosslinking procedure in which the crosslinking was interrupted after a period of time by washing away serum albumin that had not yet deposited on the silica. Subsequently, more glutaraldehyde was added to ensure that the remaining albumin was tightly crosslinked and permanently attached to the silica. The two stage process ensured that large clumps of support particles were not glued together. Such clumps disrupt flow through the column and degrade efficiency. See, e.g., R. A. Thompson et al, ". . . Sorbents Obtained by Entrapment of Crosslinked Bovine Serum Albumin in Silica", Journal Chromatography, Vol. 465 (1989) pp. 263-270.

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Reverse phase, cation exchange, and anion exchange organic **resin or silica**-based supports are sufficiently protein-absorptive to be used, as is untreated silica. The preferred porous protein-adsorptive support is a porous silica support such as porous silica having a pore diameter of 30 to 300 .ANG., and a particle size of 1 to 500 micrometers; although, any protein-adsorptive support may be used. When a porous silica support is used it may be one already having an alkylsilane bonded to the surfaces thereof or it may be a dual zone or mixed phase material such as that shown in my U.S. Pat. Nos. 4,773,994, 4,778,600, 4,782,040, 4,950,634 and 4,950,635. In all instances, the coating of crosslinked protein covers the external surfaces of the porous silica support overcoating the alkyl or ketal-blocked-diol or fluoroalkyl or other external phase of the dual zone or mixed phase material.

CCOR:
210/198.2

DOCUMENT-IDENTIFIER: US 5470463 A
TITLE: Passivated porous supports and methods for the preparation and use of
same

BSPR:
Several previous investigators have sought to passivate various microporous
media including membranes and particulate chromatographic supports by applying
thin surface coatings to inorganic or organic/polymeric substrates. For
example, Steuck, in U.S. Pat. No. 4,618,533, discloses a porous polymeric
membrane substrate fashioned from a thermoplastic organic polymer upon which a
permanent coating is grafted and/or deposited on the entire membrane surface.
The polymerization and crosslinking of the polymerizable monomer upon and
within the porous membrane substrate is performed in such a way that a thin
coating is deposited upon the entire surface of the porous membrane, including
the inner pore walls. Significantly, the porous configurations of the coated,
composite membrane structures claimed by Steuck are essentially identical to
those of the corresponding uncoated porous membrane substrates, implying that
the polymer of Steuck is applied as a thin surface layer or coating that does
not interfere with the porosity or flow properties of his **composite** membranes.
Moreover, Steuck does not disclose the concept of a "passivating layer" or the
use of monomers capable of functioning as "passivating" monomers within the
meaning of the present invention as discussed in more detail below.

BSPR:
While it has been discovered that this process of depositing protective polymer
coatings upon the porous surfaces of mineral oxide (and particularly silica)
matrices can significantly stabilize these materials by sharply reducing their

rates of chemical leaching, the approach has the important disadvantage of rendering the porous surfaces of the coated and protected matrices hydrophobic and thus prone to cause excessive non-specific binding of proteins by adsorption. (This is precisely the same problem noted above in connection with entirely polymeric porous support matrices.) However, this problem can be successfully addressed by the methods of the present invention in the same way as the non-specific binding of strictly polymeric support matrices can be reduced--i.e., by passivation in a process of oriented polymerization. More particularly, these **composite** chromatographic supports (i.e., supports comprised of mineral oxide substrates that have been stabilized by the application of thin protective polymer coatings) can be passivated against excessive non-specific binding by incorporating passivating ("neutralizing") monomers capable of associating with and consequently deactivating innate nonpolar hydrophobic groups exposed on the matrix surface. The passivating monomers useful in this embodiment of the present invention adsorb upon (and consequently cover) the hydrophobic groups on the surface by virtue of their containing long-chain saturated hydrocarbons, olefinic hydrocarbon groups, aromatic groups, or like hydrophobic domains that interact with and become appreciably bound to their hydrophobic counterparts on the matrix surface as a consequence of the hydrophobic-hydrophobic interaction existing between them.

Typically, the present invention utilizes base matrices having the following characteristics: an initial average particle size ranging from about 5 to about 1000 microns; an initial porous volume ranging from about 0.2 to about 2 cm.sup.3 /gram; an initial surface area ranging from about 1 to about 800 m.sup.2 /gram; and an initial pore size ranging from about 50 to about 6000

angstroms. Preferably, the base matrix is characterized by: an initial average particle size ranging from about 10 to about 300 microns, although passivated supports having narrow particle size ranges, such as about 15-20, about 15-25, about 30-45, about 50-60, about 80-100, and about 100-300 microns, are most preferred. Preferred ranges for other characteristics include an initial porous volume ranging from about 0.8 to about 1.2 cm.sup.3 /gram; an initial surface area ranging from about 10 to about 400 m.sup.2 /gram; and an initial pore size ranging from about 1000 to about 3000 angstroms. The density of the porous solid matrix obviously varies with its chemical nature, being higher for mineral oxide (e.g., silica) substrates and lower for polymeric ones (e.g., polystyrene).

DEPR:
It is thus demonstrated that the level of nonspecific adsorption for lysozyme (a strong cationic protein) is high when the MAPTAC is absent. The non-specific adsorption for silica with large surface areas (X 075, 100 m.sup.2 /g) is higher (55 mg/ml of **resin) than the non-specific adsorption for silica** X 015 (25 m.sup.2 /g; 15 mg/ml of resin). A certain proportionality exists between the surface area and the original level of non-specific absorptions. The amount of MAPTAC to decrease the level of non-specific adsorption down to zero is also proportional to the surface area available: 1.5% of MAPTAC is necessary with silica X 015 (25 m.sup.2 /g) whereas at least 6% is necessary to passivate silica X 075 (110 m.sup.2 /g).

DEPR:
The passivated resin is then recovered by filtration. The oil is eliminated with an extensive washing with water containing 0.1-0.5% of a non-ionic detergent and then stored in a saline buffer at neutral pH. The product resin

shows very similar ion-exchange characteristics as those described in Example 2. Additionally, its sensitivity in strong alkaline media is much improved as measured by its weight loss after one night of contact with 0.5M sodium hydroxide. The passivated resin of this example lost only about half as much weight as an anionic **resin prepared from silica** having an unprotected surface area.

DEPC:
Preparation of an Anion-Exchange **Resin Using a Surface-Protected (i.e., Pre-coated) Silica** Passivated Porous Support

DEPC:
Determination of Ion-Exchange and Protein Sorption Capacity of Preparation of Anion-Exchange **Resins Based on Passivated Porous Silica** Support of Different Surface Areas

CCOR:
210/198.2

DOCUMENT-IDENTIFIER: US 5445732 A
TITLE: Passivated porous polymer supports and methods for the preparation and
use of same

BSPR:
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media including membranes and particulate chromatographic supports by applying
thin surface coatings to inorganic or organic/polymeric substrates. For
example, Steuck, in U.S. Pat. No. 4,618,533, discloses a porous polymeric
membrane substrate fashioned from a thermoplastic organic polymer upon which a
permanent coating is grafted and/or deposited on the entire membrane surface.
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within the porous membrane substrate is performed in such a way that a thin
coating is deposited upon the entire surface of the porous membrane, including
the inner pore walls. Significantly, the porous configurations of the coated,
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those of the corresponding uncoated porous membrane substrates, implying that
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Moreover, Steuck does not disclose the concept of a "passivating layer" or the
use of monomers capable of functioning as "passivating" monomers within the
meaning of the present invention as discussed in more detail below.

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While it has been discovered that this process of depositing protective polymer
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matrices can significantly stabilize these materials by sharply reducing their

rates of chemical leaching, the approach has the important disadvantage of rendering the porous surfaces of the coated and protected matrices hydrophobic and thus prone to cause excessive non-specific binding of proteins by adsorption. (This is precisely the same problem noted above in connection with entirely polymeric porous support matrices.) However, this problem can be successfully addressed by the methods of the present invention in the same way as the non-specific binding of strictly polymeric support matrices can be reduced--i.e., by passivation in a process of oriented polymerization. More particularly, these **composite** chromatographic supports (i.e., supports comprised of mineral oxide substrates that have been stabilized by the application of thin protective polymer coatings) can be passivated against excessive non-specific binding by incorporating passivating ("neutralizing") monomers capable of associating with and consequently deactivating innate non-polar hydrophobic groups exposed on the matrix surface. The passivating monomers useful in this embodiment of the present invention adsorb upon (and consequently cover) the hydrophobic groups on the surface by virtue of their containing long-chain saturated hydrocarbons, olefinic hydrocarbon groups, aromatic groups, or like hydrophobic domains that interact with and become appreciably bound to their hydrophobic counterparts on the matrix surface as a consequence of the hydrophobic-hydrophobic interaction existing between them.

Typically, the present invention utilizes base matrices having the following characteristics: an initial average particle size ranging from about 5 to about 1000 microns; an initial porous volume ranging from about 0.2 to about 2 cm.sup.3 /gram; an initial surface area ranging from about 1 to about 800 m.sup.2 /gram; and an initial pore size ranging from about 50 to about 6000

angstroms. Preferably, the base matrix is characterized by: an initial average particle size ranging from about 10 to about 300 microns, although passivated supports having narrow particle size ranges, such as about 15-20, about 15-25, about 30-45, about 50-60, about 80-100, and about 100-300 microns, are most preferred. Preferred ranges for other characteristics include an initial porous volume ranging from about 0.8 to about 1.2 cm.sup.3 /gram; an initial surface area ranging from about 10 to about 400 m.sup.2 /gram; and an initial pore size ranging from about 1000 to about 3000 angstroms. The density of the porous solid matrix obviously varies with its chemical nature, being higher for mineral oxide (e.g., silica) substrates and lower for polymeric ones (e.g., polystyrene).

DEPR:
It is thus demonstrated that the level of non-specific adsorption for lysozyme (a strong cationic protein) is high when the MAPTAC is absent. The non-specific adsorption for silica with large surface ares (X 075, 100m.sup.2 /g) is higher (55 mg/ml of **resin) than the non-specific adsorption for silica** X 015 (25 m.sup.2 /g; 15 mg/ml of resin). A certain proportionality exists between the surface area and the original level of non-specific absorptions. The amount of MAPTAC to decrease the level of non-specific adsorption down to zero is also proportional to the surface area available: 1.5% of MAPTAC is necessary with silica X 015 (25 m.sup.2 /g) whereas at least 6% is necessary to passivate silica X 075 (100 m.sup.2 /g).

DEPR:
The passivated resin is then recovered by filtration. The oil is eliminated with an extensive washing with water containing 0.1-0.5% of a non-ionic detergent and then stored in a saline buffer at neutral pH. The product resin

shows very similar ion-exchange characteristics as those described in Example 2. Additionally, its sensitivity in strong alkaline media is much improved as measured by its weight loss after one night of contact with 0.5M sodium hydroxide. The passivated resin of this example lost only about half as much weight as an artionic **resin prepared from silica** having an unprotected surface area.

DEPL:
Preparation of an Anion-Exchange **Resin Using a Surface-Protected (i.e., Pre-coated) Silica** Passivated Porous Support

DEPL:
Determination of Ion-Exchange and Protein Sorption Capacity of Preparation of Artion-Exchange **Resins Based on Passivated Porous Silica** Support of Different Surface Areas

CCOR:
210/198.2

(5)

DOCUMENT-IDENTIFIER: US RE34910 E
TITLE: Carbon-clad zirconium oxide particles

BSPR:
Packing materials for high pressure liquid chromatography (HPLC) have also been based on carbon. For example, carbon-based supports useful for HPLC applications have included the following: graphitized carbon black (GCB), pyrocarbon reinforced GCB, and more recently, a porous graphitic carbon (PGC). PGC is prepared by filling the pores of a silica gel with a polymer comprising carbon, thermolyzing the polymer to produce a silica/carbon **composite,** dissolving out the silica to produce a porous carbon, and subjecting the porous carbon to graphitizing conditions.

BSPR:
For example, U.K. Patent Application No. 2,035,282 discloses a method for producing a porous carbon material suitable for chromatography or use as a catalyst support, which involves depositing carbon in the pores of a porous inorganic template material such as silica gel, porous glass, alumina or other porous refractory oxides having a surface area of at least 1 m.sup.2 /g, and thereafter removing the template material. O. Chiantore et al., Analytical Chemistry, 60, 638-642 (1988), disclose carbon sorbents which were prepared by pyrolysis of either phenol formaldehyde **resin or saccharose on spheroidal silica** gels coated with these materials. The pyrolysis is performed at 600.degree. C. for one hour in an inert atmosphere, and the silica is subsequently removed by boiling the material in an excess of a 10% NaOH solution for 30 minutes. Chiantore et al. conclude that, at the temperatures employed in their work, the carbonaceous polymer network that was formed still maintained some of the chemical features of the starting material

(page 641, column 2). To obtain carbons where polar functional groups have been completely eliminated, the authors conclude that high temperatures (greater than 800.degree. C.) treatments under inert atmosphere are necessary.

BSPR:
The present invention provides a **composite** support material which is useful as a stationary phase in liquid chromatography, particularly in high-performance liquid chromatography. The **composite** support material comprises carbon-clad particles of zirconium oxide (also referred to herein as ZrO.sub.2, or as "zirconia"). In order to facilitate packing of liquid chromatography columns, it is preferred that each individual unit of the present support material be a substantially spherical particle; thus, the preferred spherical particles will be referred to herein as "spherules." However, the present invention is also intended to provide support materials useful in low performance chromatography, fluidized beds, and general batch absorbers. There is no requirement that the present particles be substantially spherical in these applications, where irregularly shaped particles are typically utilized.

DEPR:
The present invention provides both a **composite** material useful as a chromatographic support, and a method for forming a chromatographic support material.

CCOR:
210/198.2

DOCUMENT-IDENTIFIER: US 5393430 A
TITLE: Passivated and stabilized porous mineral oxide supports and methods for
the preparation and use of same

BSPR:
Several previous investigators have sought to passivate various microporous
media including membranes and particulate chromatographic supports by applying
thin surface coatings to inorganic or organic/polymeric substrates. For
example, Steuck, in U.S. Pat. No. 4,618,533, discloses a porous polymeric
membrane substrate fashioned from a thermoplastic organic polymer upon which a
permanent coating is grafted and/or deposited on the entire membrane surface.
The polymerization and crosslinking of the polymerizable monomer upon and
within the porous membrane substrate is performed in such a way that a thin
coating is deposited upon the entire surface of the porous membrane, including
the inner pore walls. Significantly, the porous configurations of the coated,
composite membrane structures claimed by Steuck are essentially identical to
those of the corresponding uncoated porous membrane substrates, implying that
the polymer of Steuck is applied as a thin surface layer or coating that does
not interfere with the porosity or flow properties of his **composite** membranes.
Moreover, Steuck does not disclose the concept of a "passivating layer" or the
use of monomers capable of functioning as "passivating" monomers within the
meaning of the present invention as discussed in more detail below.

BSPR:
While it has been discovered that this process of depositing protective polymer
coatings upon the porous surfaces of mineral oxide (and particularly silica)
matrices can significantly stabilize these materials by sharply reducing their

rates of chemical leaching, the approach has the important disadvantage of rendering the porous surfaces of the coated and protected matrices hydrophobic and thus prone to cause excessive non-specific binding of proteins by adsorption. (This is precisely the same problem noted above in connection with entirely polymeric porous support matrices.) However, this problem can be successfully addressed by the methods of the present invention in the same way as the non-specific binding of strictly polymeric support matrices can be reduced--i.e., by passivation in a process of oriented polymerization. More particularly, these **composite** chromatographic supports (i.e., supports comprised of mineral oxide substrates that have been stabilized bythe application of thin protective polymer coatings) can be passivated against excessive non-specific binding by incorporating passivating ("neutralizing") monomers capable of associating with and consequently deactivating innate non-polar hydrophobic groups exposed on the matrix surface. The passivating monomers useful in this embodiment of the present invention adsorb upon (and consequently cover) the hydrophobic groups on the surface by virtue of their containing long-chain saturated hydrocarbons, olefinic hydrocarbon groups, aromatic groups, or like hydrophobic domains that interact with and become appreciably bound to their hydrophobic counterparts on the matrix surface as a consequence of the hydrophobic-hydrophobic interaction existing between them.

Typically, the present invention utilizes. base matrices having the following characteristics: an initial average particle size ranging from about 5 to about 1000 microns; an initial porous volume ranging from about 0.2 to about 2 cm.sup.3 /gram; an initial surface area ranging from about 1 to about 800 m.sup.2 /gram; and an initial pore size ranging from about 50 to about 6000

angstroms. Preferably, the base matrix is characterized by: an initial average particle size ranging from about 10 to about 300 microns, although passivated supports having narrow particle size ranges, such as about 15-20, about 15-25, about 30-45, about 50-60, about 80-100, and about 100-300 microns, are most preferred. Preferred ranges for other characteristics include an initial porous volume ranging from about 0.8 to about 1.2 cm.sup.3 /gram; an initial surface area ranging from about 10 to about 400 m.sup.2 /gram; and an initial pore size ranging from about 1000 to about 3000 angstroms. The density of the porous solid matrix obviously varies with its chemical nature, being higher for mineral oxide (e.g., silica) substrates and lower for polymeric ones (e.g., polystyrene).

DEPR:
It is thus demonstrated that the level of non-specific adsorption for lysozyme (a strong cationic protein) is high when the MAPTAC is absent. The non-specific adsorption for silica with large surface ares (X 075, 100 m.sup.2 /g) is higher (55 mg/ml of **resin) than the non-specific adsorption for silica** X 015 (25 m.sup.2 /g; 15 mg/ml of resin). A certain proportionality exists between the surface area and the original level of non-specific absorptions. The amount of MAPTAC to decrease the level of non-specific adsorption down to zero is also proportional to the surface area available: 1.5% of MAPTAC is necessary with silica X 015 (25 m.sup.2 /g) whereas at least 6% is necessary to passivate silica X 075 (100 m.sup.2 /g).

DEPR:
The passivated resin is then recovered by filtration. The oil is eliminated with an extensive washing with water containing 0.1-0.5% of a non-ionic detergent and then stored in a saline buffer at neutral pH. The product resin

shows very similar ion-exchange characteristics as those described in Example 2. Additionally, its sensitivity in strong alkaline media is much improved as measured by its weight loss after one night of contact with 0.5M sodium hydroxide. The passivated resin of this example lost only about half as much weight as an artionic **resin prepared from silica** having an unprotected surface area.

DEPL:
Preparation of an Anion-Exchange **Resin Using a Surface-Protected (i.e., Pre-coated) Silica** Passivated Porous Support

DEPL:
Determination of Ion-Exchange and Protein Sorption Capacity of Preparation of Anion-Exchange **Resins Based on Passivated Porous Silica** Support of Different Surface Areas

CCXR:
210/198.2

DOCUMENT-IDENTIFIER: US 5346619 A
TITLE: Carbon-clad zirconium oxide particles

BSPR:
Packing materials for high pressure liquid chromatography (HPLC) have also been based on carbon. For example, carbon-based supports useful for HPLC applications have included the following: graphitized carbon black (GCB), pyrocarbon reinforced GCB, and more recently, a porous graphitic carbon (PGC). PGC is prepared by filling the pores of a silica gel with a polymer comprising carbon, thermolyzing the polymer to produce a silica/carbon **composite,** dissolving out the silica to produce a porous carbon, and subjecting the porous carbon to graphitizing conditions.

BSPR:
For example, U.K. Patent Application No. 2,035,282 discloses a method for producing a porous carbon material suitable for chromatography or use as a catalyst support, which involves depositing carbon in the pores of a porous inorganic template material such as silica gel, porous glass, alumina or other porous refractory oxides having a surface area of at least 1 m.sup.2 /g, and thereafter removing the template material. O. Chiantore et al., Analytical Chemistry, 60, 638-642 (1988), disclose carbon sorbents which were prepared by pyrolysis of either phenol formaldehyde **resin or saccharose on spheroidal silica** gels coated with these materials. The pyrolysis is performed at 600.degree. C. for one hour in an inert atmosphere, and the silica is subsequently removed by boiling the material in an excess of a 10% NaOH solution for 30 minutes. Chiantore et al. conclude that, at the temperatures employed in their work, the carbonaceous polymer network that was formed still maintained some of the chemical features of the starting material

(page 641, column 2). To obtain carbons where polar functional groups have been completely eliminated, the authors conclude that high temperatures (greater than 800.degree. C.) treatments under inert atmosphere are necessary.

BSPR:
The present invention provides a **composite** support material which is useful as a stationary phase in liquid chromatography, particularly in high-performance liquid chromatography. The **composite** support material comprises carbon-clad particles of zirconium oxide (also referred to herein as ZrO.sub.2, or as "zirconia"). In order to facilitate packing of liquid chromatography columns, it is preferred that each individual unit of the present support material be a substantially spherical particle; thus, the preferred spherical particles will be referred to herein as "spherules". However, the present invention is also intended to provide support materials useful in low performance chromatography, fluidized beds, and general batch absorbers. There is no requirement that the present particles be substantially spherical in these applications, where irregularly shaped particles are typically utilized.

DEPR:
The present invention provides both a **composite** material useful as a chromatographic support, and a method for forming a chromatographic support material.

CCOR:
210/198.2

DOCUMENT-IDENTIFIER: US 5271833 A
TITLE: Polymer-coated carbon-clad inorganic oxide particles

BSPR:
Packing materials for high pressure liquid chromatography (HPLC) have also been based on carbon. For example, carbon-based supports useful for HPLC applications have included the following: graphitized carbon black (GCB), pyrocarbon reinforced GCB, and more recently, a porous graphitic carbon (PGC). PGC is prepared by filling the pores of a silica gel with a polymer comprising carbon, thermolyzing the polymer to produce a silica/carbon **composite,** dissolving out the silica to produce a porous carbon, and subjecting the porous carbon to graphitizing conditions.

BSPR:
For example, U.K. Patent Application No. 2,035,282 discloses a method for producing a porous carbon material suitable for chromatography or use as a catalyst support, which involves depositing carbon in the pores of a porous inorganic template material such as silica gel, porous glass, alumina or other porous refractory oxides having a surface area of at least 1 m.sup.2 /g, and thereafter removing the template material. O. Chiantore et al., Analytical Chemistry, 60, 638-642 (1988), disclose carbon sorbents which were prepared by pyrolysis of either phenol formaldehyde **resin or saccharose on spheroidal silica** gels coated with these materials. The pyrolysis is performed at 600.degree. C. for one hour in an inert atmosphere, and the silica is subsequently removed by boiling the material in an excess of a 10% NaOH solution for 30 minutes. Chiantore et al. conclude that, at the temperatures employed in their work, the carbonaceous polymer network that was formed still maintained some of the chemical features of the starting material

(page 641, column 2). To obtain carbons where polar functional groups have been completely eliminated, the authors conclude that high temperatures (greater than 800.degree. C.) treatments under inert atmosphere are necessary.

BSPR:
The present invention provides a **composite** support material which is useful as a stationary phase in liquid chromatography, particularly in high-performance liquid chromatography. The **composite** support material comprises carbon-clad particles of zirconium oxide (also referred to herein as ZrO.sub.2, or as "zirconia"). In order to facilitate packing of liquid chromatography columns, it is preferred that each individual unit of the present support material be a substantially spherical particle; thus, the preferred spherical particles will be referred to herein as "spherules." However, the present invention is also intended to provide support materials useful in low performance chromatography, fluidized beds, and general batch absorbers. There is no requirement that the present particles be substantially spherical in these applications, where irregularly shaped particles are typically utilized.

DEPR:
The present invention provides both a **composite** material useful as a chromatographic support, and a method for forming a chromatographic support material.

CCOR:
210/198.2

DOCUMENT-IDENTIFIER: US 5270280 A
TITLE: Packing material for liquid chromatography and method of manufacturing
thereof

BSPR:
As a packing material for liquid chromatography, there are conventionally used a chemically bonded type of packing material based on **silica gel and a packing material based on synthetic resin**. The silica gel-based packing material is relatively strong in mechanical strength and is small in swelling/shrinking characteristics against various organic solvents. Therefore, it has a high resolving power and is superior in exchangeability of eluent for analysis.

BSPR:
The granulated particles obtained are **composite** bodies comprising carbon black and binder. In the first aspect of this invention, the **composite** bodies are heated to 800.degree. C. to 3000.degree. C. for graphitization (carbonization) of the binder, and consequently a packing material for liquid chromatography of this invention is obtained. If the graphitization (carbonization) temperature is below 800.degree. C., the graphitization (carbonization) of the binder is not sufficient, resulting in an insufficient strength of the packing material. If the graphitization (carbonization) temperature is above 3000.degree. C., it is not preferable in that the graphitization (carbonization) yield is remarkably lowered and that the strength of the packing material is decreased. This graphitizing (carbonizing) treatment is carried out in an inert gas or under vacuum. It is preferable, prior to the graphitizing (carbonizing) treatment, to first heat the granulated particles to about 150.degree. C. to evaporate the organic binding agent in the **composite** particles and then to heat them to about

500.degree. C. to
harden and infusibilize the binder.

CCXR:
210/198.2

DOCUMENT-IDENTIFIER: US 5268097 A
TITLE: Passivated and stabilized porous mineral oxide supports and method for
the preparation and use of same

BSPR:
Several previous investigators have sought to passivate various microporous
media including membranes and particulate chromatographic supports by applying
thin surface coatings to inorganic or organic/polymeric substrates. For
example, Steuck, in U.S. Pat. No. 4,618,533, discloses a porous polymeric
membrane substrate fashioned from a thermoplastic organic polymer upon which a
permanent coating is grafted and/or deposited on the entire membrane surface.
The polymerization and crosslinking of the polymerizable monomer upon and
within the porous membrane substrate is performed in such a way that a thin
coating is deposited upon the entire surface of the porous membrane, including
the inner pore walls. Significantly, the porous configurations of the coated,
composite membrane structures claimed by Steuck are essentially identical to
those of the corresponding uncoated porous membrane substrates, implying that
the polymer of Steuck is applied as a thin surface layer or coating that does
not interfere with the porosity or flow properties of his **composite** membranes.
Moreover, Steuck does not disclose the concept of a "passivating layer" or the
use of monomers capable of functioning as "passivating" monomers within the
meaning of the present invention as discussed in more detail below.

BSPR:
While it has been discovered that this process of depositing protective polymer
coatings upon the porous surfaces of mineral oxide (and particularly silica)
matrices can significantly stabilize these materials by sharply reducing their

rates of chemical leaching, the approach has the important disadvantage of rendering the porous surfaces of the coated and protected matrices hydrophobic and thus prone to cause excessive non-specific binding of proteins by adsorption. (This is precisely the same problem noted above in connection with entirely polymeric porous support matrices.) However, this problem can be successfully addressed by the methods of the present invention in the same way as the non-specific binding of strictly polymeric support matrices can be reduced--i.e., by passivation in a process of oriented polymerization. More particularly, these **composite** chromatographic supports (i.e., supports comprised of mineral oxide substrates that have been stabilized by the application of thin protective polymer coatings) can be passivated against excessive non-specific binding by incorporating passivating ("neutralizing") monomers capable of associating with and consequently deactivating innate non-polar hydrophobic groups exposed on the matrix surface. The passivating monomers useful in this embodiment of the present invention adsorb upon (and consequently cover) the hydrophobic groups on the surface by virtue of their containing long-chain saturated hydrocarbons, olefinic hydrocarbon groups, aromatic groups, or like hydrophobic domains that interact with and become appreciably bound to their hydrophobic counterparts on the matrix surface as a consequence of the hydrophobic-hydrophobic interaction existing between them.

Typically, the present invention utilizes base matrices having the following characteristics: an initial average particle size ranging from about 5 to about 1000 microns; an initial porous volume ranging from about 0.2 to about 2 cm.sup.3 /gram; an initial surface area ranging from about I to about 800 m.sup.2 /gram; and an initial pore size ranging from about 50 to about 6000

angstroms. Preferably, the base matrix is characterized by: an initial average particle size ranging from about 10 to about 300 microns, although passivated supports having narrow particle size ranges, such as about 15-20, about 15-25, about 30-45, about 50-60, about 80-100, and about 100-300 microns, are most preferred. Preferred ranges for other characteristics include an initial porous volume ranging from about 0.8 to about 1.2 cm.sup.3 /gram; an initial surface area ranging from about 10 to about 400 m.sup.2 /gram; and an initial pore size ranging from about 1000 to about 3000 angstroms. The density of the porous solid matrix obviously varies with its chemical nature, being higher for mineral oxide (e.g., silica) substrates and lower for polymeric ones (e.g., polystyrene).

DEPR:
It is thus demonstrated that the level of non-specific adsorption for lysozyme (a strong cationic protein) is high when the MAPTAC is absent. The non-specific adsorption for silica with large surface ares (X 075, 100 m.sup.2 /g) is higher (55 mg/ml of **resin) than the non-specific adsorption for silica** X 015 (25 m.sup.2 /g; 15 mg/ml of resin). A certain proportionality exists between the surface area and the original level of non-specific absorptions. The amount of MAPTAC to decrease the level of non-specific adsorption down to zero is also proportional to the surface area available: 1.5% of MAPTAC is necessary with silica X 015 (25 m.sup.2 /g) whereas at least 6% is necessary to passivate silica X 075 (100 m.sup.2 /g).

DEPR:
The passivated resin is then recovered by filtration. The oil is eliminated with an extensive washing with water containing 0.1-0.5% of a non-ionic detergent and then stored in a saline buffer at neutral pH. The product resin

shows very similar ion-exchange characteristics as those described in Example 2. Additionally, its sensitivity in strong alkaline media is much improved as measured by its weight loss after one night of contact with 0.5M sodium hydroxide. The passivated resin of this example lost only about half as much weight as an artionic **resin prepared from silica** having an unprotected surface area.

DEPC:
Preparation of an Anion-Exchange **Resin Using a Surface-Protected (i.e., Pre-coated) Silica** Passivated Porous Support

DEPC:
Determination of Ion-Exchange and Protein Sorption Capacity of Preparation of Anion-Exchange **Resins Based on Passivated Porous Silica** Support of Different Surface Areas

CCOR:
210/198.2

(6)

DOCUMENT-IDENTIFIER: US 5182016 A
TITLE: Polymer-coated carbon-clad inorganic oxide particles

BSPR:
Packing materials for high pressure liquid chromatography (HPLC) have also been based on carbon. For example, carbon-based supports useful for HPLC applications have included the following: graphitized carbon black (GCB), pyrocarbon reinforced GCB, and more recently, a porous graphitic carbon (PGC). PGC is prepared by filling the pores of a silica gel with a polymer comprising carbon, thermolyzing the polymer to produce a silica/carbon **composite,** dissolving out the silica to produce a porous carbon, and subjecting the porous carbon to graphitizing conditions.

BSPR:
For example, U.K. Patent Application No. 2,035,282 discloses a method for producing a porous carbon material suitable for chromatography or use as a catalyst support, which involves depositing carbon in the pores of a porous inorganic template material such as silica gel, porous glass, alumina or other porous refractory oxides having a surface area of at least 1 m.sup.2 /g, and thereafter removing the template material. O. Chiantore et al., Analytical Chemistry, 60, 638-642 (1988), disclose carbon sorbents which were prepared by pyrolysis of either phenol formaldehyde **resin or saccharose on spheroidal silica** gels coated with these materials. The pyrolysis is performed at 600.degree. C. for one hour in an inert atmosphere, and the silica is subsequently removed by boiling the material in an excess of a 10% NaOH solution for 30 minutes. Chiantore et al. conclude that, at the temperatures employed in their work, the carbonaceous polymer network that was formed still maintained some of the chemical features of the starting material

(page 641, column 2). To obtain carbons where polar functional groups have been completely eliminated, the authors conclude that high temperatures (greater than 800.degree. C.) treatments under inert atmosphere are necessary.

BSPR:
The present invention provides a **composite** support material which is useful as a stationary phase in liquid chromatography, particularly in high-performance liquid chromatography. The **composite** support material comprises carbon-clad particles of zirconium oxide (also referred to herein as ZrO.sub.2, or as "zirconia"). In order to facilitate packing of liquid chromatography columns, it is preferred that each individual unit of the present support material be a substantially spherical particle; thus, the preferred spherical particles will be referred to herein as "spherules." However, the present invention is also intended to provide support materials useful in low performance chromatography, fluidized beds, and general batch absorbers. There is no requirement that the present particles be substantially spherical in these applications, where irregularly shaped particles are typically utilized.

DEPR:
The present invention provides both a **composite** material useful as a chromatographic support, and a method for forming a chromatographic support material.

CCOR:
210/198.2

DOCUMENT-IDENTIFIER: US 5135649 A
TITLE: Column packing material with both hydrophobic and hydrophilic groups and
process for production thereof

DEPR:
As the porous support to be used in the present invention, there can be
employed, for example, silica gel, alumina, glass beads (e.g., porous glass),
zeolite, hydroxyapatite, or graphite; namely, any powder generally employed as
the support for chromatography. Also, a **composite** powder, such as a powder
coated with a fine inorganic powder, for example, **silica gel, titanium oxide or
hydroxyapatite, on the surface of a synthetic resin** such as polyamide, acrylic
resin, or polyvinyl alcohol, can be employed.

CLPR:
3. A column packing material as claimed in claim 1, wherein the porous support
is a **composite** powder comprising a synthetic resin coated with a fine inorganic
powder.

CCOR:
210/198.2

DOCUMENT-IDENTIFIER: US 5108597 A
TITLE: Carbon-clad zirconium oxide particles

BSPR:
Packing materials for high pressure liquid chromatography (HPLC) have also been based on carbon. For example, carbon-based supports useful for HPLC applications have included the following: graphitized carbon black (GCB), pyrocarbon reinforced GCB, and more recently, a porous graphitic carbon (PGC). PGC is prepared by filling the pores of a silica gel with a polymer comprising carbon, thermolyzing the polymer to produce a silica/carbon **composite,** dissolving out the silica to produce a porous carbon, and subjecting the porous carbon to graphitizing conditions.

BSPR:
For example, U.K. Patent Application No. 2,035,282 discloses a method for producing a porous carbon material suitable for chromatography or use as a catalyst support, which involves depositing carbon in the pores of a porous inorganic template material such as silica gel, porous glass, alumina or other porous refractory oxides having a surface area of at least 1 m.sup.2 /g, and thereafter removing the template material. O. Chiantore et al., Analytical Chemistry, 60, 638-642 (1988), disclose carbon sorbents which were prepared by pyrolysis of either phenol formaldehyde **resin or saccharose on spheroidal silica** gels coated with these materials. The pyrolysis is performed at 600.degree. C. for one hour in an inert atmosphere, and the silica is subsequently removed by boiling the material in an excess of a 10% NaOH solution for 30 minutes. Chiantore et al. conclude that, at the temperatures employed in their work, the carbonaceous polymer network that was formed still maintained some of the chemical features of the starting material

(page 641, column 2). To obtain carbons where polar functional groups have been completely eliminated, the authors conclude that high temperatures (greater than 800.degree. C) treatments under inert atmosphere are necessary.

BSPR:
The present invention provides a **composite** support material which is useful as a stationary phase in liquid chromatography, particularly in high-performance liquid chromatography. The **composite** support material comprises carbon-clad particles of zirconium oxide (also referred to herein as ZrO.sub.2, or as "zirconia"). In order to facilitate packing of liquid chromatography columns, it is preferred that each individual unit of the present support material be a substantially spherical particle; thus, the preferred spherical particles will be referred to herein as "spherules." However, the present invention is also intended to provide support materials useful in low performance chromatography, fluidized beds, and general batch absorbers. There is no requirement that the present particles be substantially spherical in these applications, where irregularly shaped particles are typically utilized.

DEPR:
The present invention provides both a **composite** material useful as a chromatographic support, and a method for forming a chromatographic support material.

CCOR:
210/198.2

DOCUMENT-IDENTIFIER: US 4314823 A
TITLE: Combination apparatus and method for chromatographic separation and
quantitative analysis of multiple ionic species

DEPR:
As set out above, the ion exclusion resin for anion separation is preferably in the hydrogen ion form. This permits conversion of the weak acids to their unionized molecular form. In addition, it causes stripping of cations which could provide interference background in conductivity cell 18. However, if desired for specialty applications, non-ionic resin may be employed, so long as the counterions in the sample are preconverted to acid form for the separation of anions or base form for the separation of cations. This is preferably performed by using a strong acid or strong base eluent. Suitable non-ionic **resins are so-called silica based reverse phase resins**. Specific suitable ones are sold under the trade designation .mu.-BondaPak C-18 by Waters Associates.

DEPR:
The use of a concentrator column in the present system increases its versatility. Thus, it permits the concentration of ionic species in the absence of its accompanying solution so that upon removal with the developing reagent, there is a minimum disturbance of the equilibrium of the separation column. In addition, if desired, multiple injections of the same sample may be made to supply multiple quantities of ionic species on the concentrator column to be resolved as a **composite** sample on ion chromatography column 53. This is particularly useful for trace quantities of an ionic species which may be difficult to resolve using a single sample. Also, by use of multiple concentrator columns (not shown) and/or appropriate adjustment of the valving,

it is possible to resolve predetermined highly specific peaks by repeated runs of preselected ionic species. Thus, although the system has been described in terms of using ion chromatography to resolve only the strong acids or bases which pass through the void volume peak by the ion chromatography stage, it should be understood that the weak ionic species which are at least partially resolved during ion exclusion on column 37 may be further resolved by ion chromatography in column 50. This may be done by loading such ionic species on the illustrated single concentrator column or by the use of multiple concentrator columns (not shown) in parallel and by appropriate adjustment in the valving. Thus, since ion exclusion and ion chromatography resolve the ionic species by different mechanisms, it may be possible that two weak acid or base ionic species may be difficult to fully resolve by ion exclusion alone but may be fully resolved by a combination of ion exclusion chromatography on separation column 37 and ion chromatography on separation column 53. One or more concentrator columns 49 facilitate this viewing or preselected peaks of ionic species.

CCXR:
210/198.2

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Date: 08/03/2000

Time: 09:53

Document Listing

Document	Image pages	Text pages	Error pages
US 6063283 A	0	1	0
US 5738783 A	0	1	0
US 5135649 A	0	1	0
Total	0	3	0

(7)

DOCUMENT-IDENTIFIER: US 6063283 A
TITLE: Method for analyzing a sample by using a liquid chromatograph

DEPR:
In the present invention, porous media commonly used in liquid chromatography
such as silica gel, alumina, porous glass beads, zeolite, hydroxyapatite and
graphite may be used as the packing material of the column 40. Alternatively,
it is possible to use **composite** powders formed of a **resin core covered by**
inorganic powders such as silica gel, titanium dioxide, hydroxyapatite, and the
like. On may use materials such as polyamides, acrylic resins, polyvinyl
alcohols, and the like, for the resin core.

CCOR:
210/656

CCXR:
210/198.2

DOCUMENT-IDENTIFIER: US 5738783 A
TITLE: Liquid chromatograph having a micro- and semi-micro column

DEPR:
In the present invention, porous media commonly used in liquid chromatography
such as silica gel, alumina, porous glass beads, zeolite, hydroxyapatite and
graphite may be used as the packing material of the column 40. Alternatively,
it is possible to use **composite** powders formed of a **resin core covered by**
inorganic powders such as silica gel, titanium dioxide, hydroxyapatite, and the
like. On may use materials such as polyamides, acrylic resins, polyvinyl
alcohols, and the like, for the resin core.

CCOR:
210/198.2

CCXR:
210/502.1

CCXR:
210/635

CCXR:
210/656

DOCUMENT-IDENTIFIER: US 5135649 A
TITLE: Column packing material with both hydrophobic and hydrophilic groups and
process for production thereof

DEPR:
As the porous support to be used in the present invention, there can be
employed, for example, silica gel, alumina, glass beads (e.g., porous glass),
zeolite, hydroxyapatite, or graphite; namely, any powder generally employed as
the support for chromatography. Also, a **composite** powder, such as a powder
coated with a fine inorganic powder, for example, **silica gel, titanium oxide or
hydroxyapatite, on the surface of a synthetic resin** such as polyamide, acrylic
resin, or polyvinyl alcohol, can be employed.

CCOR:
210/198.2

CCXR:
210/502.1

CCXR:
210/635

CCXR:
210/656

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UserID: ETherkorn

Computer: WS08186

Date: 08/03/2000

Time: 09:55

Document Listing

Document	Image pages	Text pages	Error pages
US 5738783 A	0	1	0
US 5135649 A	0	1	0
Total	0	2	0

DOCUMENT-IDENTIFIER: US 5738783 A
TITLE: Liquid chromatograph having a micro- and semi-micro column

DEPR:
In the present invention, porous media commonly used in liquid chromatography
such as silica gel, alumina, porous glass beads, zeolite, hydroxyapatite and
graphite may be used as the packing material of the column 40. Alternatively,
it is possible to use **composite** powders formed of a **resin core covered by**
inorganic powders such as silica gel, titanium dioxide, hydroxyapatite, and the
like. On may use materials such as polyamides, acrylic resins, polyvinyl
alcohols, and the like, for the resin core.

CCXR:
210/635

DOCUMENT-IDENTIFIER: US 5135649 A
TITLE: Column packing material with both hydrophobic and hydrophilic groups and
process for production thereof

DEPR:
As the porous support to be used in the present invention, there can be
employed, for example, silica gel, alumina, glass beads (e.g., porous glass),
zeolite, hydroxyapatite, or graphite; namely, any powder generally employed as
the support for chromatography. Also, a **composite** powder, such as a powder
coated with a fine inorganic powder, for example, **silica gel, titanium oxide or
hydroxyapatite, on the surface of a synthetic resin** such as polyamide, acrylic
resin, or polyvinyl alcohol, can be employed.

CCXR:
210/635

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Computer: WS08186

Date: 08/03/2000

Time: 09:57

Document Listing

Document	Image pages	Text pages	Error pages
US 5738783 A	0	1	0
US 5135649 A	0	1	0
Total	0	2	0

DOCUMENT-IDENTIFIER: US 5738783 A
TITLE: Liquid chromatograph having a micro- and semi-micro column

DEPR:
In the present invention, porous media commonly used in liquid chromatography
such as silica gel, alumina, porous glass beads, zeolite, hydroxyapatite and
graphite may be used as the packing material of the column 40. Alternatively,
it is possible to use **composite** powders formed of a **resin core covered by**
inorganic powders such as silica gel, titanium dioxide, hydroxyapatite, and the
like. On may use materials such as polyamides, acrylic resins, polyvinyl
alcohols, and the like, for the resin core.

CCXR:
210/502.1

DOCUMENT-IDENTIFIER: US 5135649 A
TITLE: Column packing material with both hydrophobic and hydrophilic groups and
process for production thereof

DEPR:
As the porous support to be used in the present invention, there can be
employed, for example, silica gel, alumina, glass beads (e.g., porous glass),
zeolite, hydroxyapatite, or graphite; namely, any powder generally employed as
the support for chromatography. Also, a **composite** powder, such as a powder
coated with a fine inorganic powder, for example, **silica gel, titanium oxide or
hydroxyapatite, on the surface of a synthetic resin** such as polyamide, acrylic
resin, or polyvinyl alcohol, can be employed.

CCXR:
210/502.1

Printed by EAST

UserID: ETherkorn

Computer: WS08186

Date: 08/03/2000

Time: 10:02

Document Listing

Document	Image pages	Text pages	Error pages
US 5997887 A	0	3	0
US 5393362 A	0	1	0
US 5238810 A	0	1	0
US 5190795 A	0	1	0
Total	0	6	0

DOCUMENT-IDENTIFIER: US 5997887 A
TITLE: Skin care compositions and method of improving skin appearance

BSPR:
The reflective particulate material preferably comprises particles of inorganic material comprising TiO.sub.2, ZnO, ZrO.sub.2 and combinations thereof, more preferably TiO.sub.2, ZnO and combinations thereof (combinations are intended to include particles which comprise one or more of these materials, as well as mixtures of these particulate materials) and most preferably, the particles consist essentially of TiO.sub.2. The particulate material may be a **composite,** e.g., deposited on a core or mixed with other materials such as, but not limited to, **silica, silicone resin,** mica, and nylon.

DEPR:
The Coverage Index values of the compositions in Examples 1-3 are obtained by the following protocol: Using a collagen film having an exposed surface area of about 7 cm.sup.2 (such as IMS #1192 or equivalent available from IMS Inc. Milford Conn.) mounted in a suitable holder, apply 40 microliters (using a Microman M50 **pipette**) of product and spread evenly by hand on the film surface using 10 finger rotations. Optionally, the sample is mounted on a Zeiss SV-11 microscope (or equivalent) equipped with a 1X lens (the microscope is useful for enlarging the image which the camera is capturing; the effective magnification of this system is about 5 microns/pixel). A mounting template can optionally be employed to aid in repositioning the sample for multiple measurements. The SV-11 should be set up so that maximum light is being transmitted to the camera (e.g., a Sony 760-MD CCD 3 Camera). To insure proper positioning and a clear image, the equipment is set in the following manner.

Camera controls are set so that the Gamma and Linear matrix switches are off.
The camera control box settings are further defined as follows: Gain=0,
White/Black balance on auto, iris- auto, mode-camera, detail-12 o'clock
position, phase-0 degrees, SC-3 o'clock position, H-12 o'clock position, Color
temp -3200 K, shutter off. The camera should be allowed to warm up for 15
minutes before adjusting white and black balance. Press the button labeled
"white" to adjust the white balance, and adjust black balance by pulling the
black adjustment rod and pushing the button that says black. Computer cables
are connected to the RGB1 and Composite Sync.ports on the camera. The
microscope Iris is set to completely open and a frosted glass plate is
positioned in the microscope base for a uniformly lit field. A clear glass
plate may optionally be used to adjust the sample height. Open the Optimas 5.2
program on the computer. Use a sample cup which is partially covered with
black tape in the light path to adjust gain and offset (brightness). The
reflecting mirror at the base of the microscope is set for maximum reflection
into the microscope. The mean of the light source should be 245 to 254.5. The
STD Deviation should be less than 3. If the mean is out of specifications
check the light bulb alignment and mirror adjustment.

DEPR:
For measuring light transmission by the test product, a piece of collagen film,
having a surface area of about 7 cm.sup.2, is first pre-hydrated with distilled
water to insure flexibility. 40 microliters of test product is then dispensed
on the film (e.g., using a Microman M50 **pipette** or equivalent), and spread
evenly over its surface to produce an even film covering the surface of the
collagen (generally by lightly spreading the material by applying 10 rotations
of the finger, wearing a clean, latex finger cot, to the

material). After
waiting for a period of 5 minutes, the sample is mounted on the microscope
base. Light transmission measurements through the film and product are then
taken in the manner described for the control. Least significant differences
can be performed on the data using Fischer's LSD method. The Coverage Index is
calculated as follows: ##EQU3##

DOCUMENT-IDENTIFIER: US 5393362 A
TITLE: Method for improving adhesion to metal

BSPR:
Composites that can be used include conventional **composites** containing an adhesive **resin as described above and a filler such as silica,** glass, and the like. Commercially available **composites** include dental restorative **composites** such as P-50.TM. and P-30.TM. dental restoratives (3M).

DEPR:
Various porcelain-fusing metal surfaces were prepared using the general procedure of EXAMPLE 1 with the following exceptions: in Step 1, the metal was dry polished; in Step 2, the surface was sandblasted for 1 minute; in Step 3, the samples were rinsed with distilled water only; an additional step (Step 3a) was added between Step 3 and Step 4, which consisted of heating the samples in a Ney STAR FIRE.TM. high performance programmable porcelain furnace (#949-14-002, J. M. Ney Company, Bloomfield, Conn.) at a particular furnace temperature for the respective metal substrate as follows: REXILLIUM.TM. III (Jeneric Industries, Inc., Wallingford, Conn.) and stainless steel 316 (3M Unitek) were heated at 1051.degree. C., RX NATURELLE.TM. (Jeneric Industries) was heated at 537.degree. C. and RX SP CG.TM. (Jeneric Industries) was heated at 1093.degree. C.; in Step 4, the organosilicon compound was applied by placing one drop of the A-174 solution on the surface of the metal disk with a plastic transfer **pipet**; and in Step 5, the samples were heated in the porcelain furnace described in Step 3a at 500.degree. C. for 10 minutes.

DOCUMENT-IDENTIFIER: US 5238810 A
TITLE: Laser magnetic immunoassay method and apparatus thereof

DEPR:
The material of the non-magnetic carrier particles may be for example, acrylic polymer **resin or polystyrene resin microspheres, or non-magnetic alumina or silica** colloidal particles. Whatever the choice of material, the density of the non-magnetic carrier particles should be greater than that of the magnetic particles because during the centrifugation procedure, the non-magnetic carrier particles are caused to precipitate and the unreacted magnetic particle labeled antibody reagent is discarded as the supernatant. This desired increased density may be achieved by employing a core of some dense, non-ferrous metal such as lead in the form of an organic or inorganic **composite** material.

DEPR:
Subsequently, as shown in FIG. 25(g), an alcohol such as methanol or ethanol was added to the well X with a micro-syringe 8e or **pipette** to dissolve the Novolak resin film to fluidize the specimen. The addition of alcohol was performed preferably by firstly removing most of the liquid in the well with a micro-syringe, adding an alcohol and then adding water. In the drawing, decomposed support is schematically indicated by a symbol (.DELTA.) and reference numeral 81a. Actually, a part of the support 81 is dispersed as solid. Thus, the antigen-antibody complex 80-82-82-84 containing micro-particles of the magnetic substance 84 floated in the well X. In this case, although a heater 85 is described in the drawing, it was not used (unnecessary).

DOCUMENT-IDENTIFIER: US 5190795 A
TITLE: Method for improving adhesion to metal

BSPR:
Composites that can be used include conventional **composites** containing an adhesive **resin as described above and a filler such as silica,** glass, and the like. Commercially available **composites** include dental restorative **composites** such as P-50.TM. and P-30.TM. dental restoratives (3M).

DEPR:
Various porcelain-fusing metal surfaces were prepared using the general procedure of EXAMPLE 1 with the following exceptions: in Step 1, the metal was dry polished; in Step 2, the surface was sandblasted for 1 minute; in Step 3, the samples were rinsed with distilled water only; an additional step (Step 3a) was added between Step 3 and Step 4, which consisted of heating the samples in a Ney STAR FIRE.TM. high performance programmable porcelain furnace (#949-14-002, J. M. Ney Company, Bloomfield, Conn.) at a particular furnace temperature for the respective metal substrate as follows: REXILLIUM.TM. III (Jeneric Industries, Inc., Wallingford, Conn.) and stainless steel 316 (3M Unitek) were heated at 1051.degree. C., RX NATURELLE.TM. (Jeneric Industries) was heated at 537.degree. C. and RX SP CG.TM. (Jeneric Industries) was heated at 1093.degree. C.; in Step 4, the organosilicon compound was applied by placing one drop of the A-174 solution on the surface of the metal disk with a plastic transfer **pipet**; and in Step 5, the samples were heated in the porcelain furnace described in Step 3a at 500.degree. C. for 10 minutes.

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6	BRS	L6	159153	chromatogra$5	USPAT	2000/08/03 09:46
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8	BRS	L8	268	6 and 7	USPAT	2000/08/03 09:47
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21	BRS	L21	4	9 and 20	USPAT	2000/08/03 10:01

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